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(54) **Nucleotide sequence having a tissue specific regulatory activity on the transcription of genes in plants**

(57) The invention relates to a nucleotide sequence having a regulatory activity on the transcription of genes

in plants and relates especially to a gene involved in the resistance mechanism of plants, to fungi, more particularly to soilborne fungi of the *Fusarium* species.

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Description

[0001] The invention relates to nucleotide sequences having a tissue specific regulatory activity on the transcription of genes in plants.

[0002] The invention is also directed to nucleotide sequences and especially DNA sequences including chimeric or recombinant constructs, wherein nucleotide sequences having a regulatory activity are used to regulate especially to initiate the transcription of nucleic acid sequences of interest in determined host cells, especially plant cells. These regulatory sequences are sometimes designated as promoter fragments, a broad meaning being given to the term "promoter".

[0003] In prior international patent application WO 97/06259, the inventors have described the isolation characterization and sequencing of a nucleic acid sequence comprising the I-2 resistance gene, which gene is involved in the resistance mechanism of plants, to plant pathogens especially to fungi and more particularly to soilborne fungi of the *Fusarium* species.

[0004] Among the pathogens causing serious damages to plants, one finds the group of soilborne cortical rots and vascular wilt inducing fungi, such as *Fusarium* and *Verticillium* (Toussoun, 1981, in: *Fusarium Diseases, Biology and Taxonomy*, Nelson, Toussoun & Cook edit., Penn. State Univ. Press, 457 pp.). Said wilt inducing fungi infect the plants through the roots via direct penetration or via wounds after which the xylem vascular tissue of the plant is colonized and symptoms of infection with said fungi are wilting, browning and dying of leaves followed by plant death. Entire plants or plant parts above the point of vascular invasion of the pathogen may die within a period of some weeks after infection. The fungi usually spread internally through the xylem vessels as mycelium or conidia until the entire plant is killed. Because of the fact that those fungi are able to survive in the soil saprophytically, they become established forever once they are introduced in the field. They are distributed more or less worldwide causing tremendous losses on most species of vegetables and flowers, field crops, fruit trees, etc. Because those fungi are so widespread and so persistent in soils the only effective way of controlling said wilt inducing fungi is using resistant plant genotypes.

[0005] Most *Fusarium* species belong to the family of imperfect fungi. This class of fungi is characterized by the fact that only a vegetative stage of the fungus is known. The generative stage of those fungi has not been discovered yet. Due to the overall classification or taxonomy of fungi upon their morphological characteristics of the generative phase, one should bear in mind that fungi belonging to the class of imperfect fungi can be classified into another class once their generative stage is discovered and, subsequently, a change of classification and name can follow (Gerlach, 1981, in: *Fusarium, Diseases, Biology and Taxonomy*, Nelson, Toussoun & Cook edit., Penn. State Univ. Press, p. 413-426). Moreover, it has been observed that some *Fusarium* species can "mutate" into another species depending on the plant infected and/or the environment (Bolton & Davidson, 1972, *Can. J. Plant Sci.*, **52**, 189-196). Up to now most of the wilt inducing *Fusarium* belong to the species *Fusarium oxysporum*. Different plant species are attacked by different races or isolates of *Fusarium* (Armstrong & Armstrong, 1981, in: *Fusarium, Diseases, Biology and Taxonomy*, Nelson, Toussoun & Cook edit., Penn. State Univ. Press, p. 391-399). However, some *Fusarium* isolates can infect different plant species. Known *Fusarium* isolates are for example: *Fusarium oxysporum* f.sp. *lycopersici* (tomato), including *Fusarium oxysporum* f.sp. *lycopersici* race 2, *F. oxysporum* f.sp. *melonis* (melon), *F. oxysporum* f.sp. *batatas* (sweet potato, tobacco), *F. oxysporum* f.sp. *cepae* (onion), *F. oxysporum* f.sp. *conglutinans* (cabbage, radish), *F. oxysporum* f.sp. *cubense* (banana), *F. oxysporum* f.sp. *vasinfectum* (cotton, alfalfa, soybean, tobacco) *F. oxysporum* f.sp. *dianthii* (carnation), *F. oxysporum* f.sp. *chrysanthemi* (chrysanthemum), *F. oxysporum* f.sp. *tuberosi* (potato), *F. oxysporum* f.sp. *cyclaminis* (cyclamen), *F. oxysporum* f.sp. *nicotianae* (tobacco).

[0006] Therefore, the name of the wilt inducing fungi can change in the future.

[0007] The characterization of the I-2 resistance gene according to WO 97/06259 has enabled the inventors to identify the gene product encoded by this I-2 resistance gene, as a polypeptide which is capable of conferring to a plant susceptible to wilt inducing fungi, resistance to infection by said pathogen(s) and/or resistance to the disease induced by said pathogen, after said plant has been transformed with the I-2 gene. The nature of resistance conferred can vary upon different factors and can encompass preventing the infection by the plant pathogen and/or as example using a signal from the invading pathogen for the activation of a defense mechanism by a related gene.

[0008] In prior international patent application, several cosmid clones including cosmid designated under B22 and A55 have been described and it has been observed that the DNA segment containing the I-2 resistance gene would encompass a region of approximately 8 kb in size. The B22 cosmid having a 17 kb size which contains said gene under a functional form, has been deposited on July 14, 1995 as plasmid pKGI2-B22 at Centraal Bureau voor Schimmelcultures at Baarn (The Netherlands) under No. CBS546.95. The A55 cosmid was deposited at the same Collection on August 5, 1996 as plasmid pKGI2-A55 under No. CBS 820.96.

[0009] In addition, in said prior international patent application, the inventors have identified that a 1.3 kb DNA non coding fragment, upstream from the I-2 translation initiation codon (ATG) located at position 1 on SEQ ID NO:4 would be sufficient to drive the transcription of the I-2 resistance gene in plants transformed with said gene. By "sufficient", it is meant that transcripts are obtained when the coding sequence is placed under the control of said 1.3 kb fragment.

[0010] The 1.3 kb fragment is identified as a 1333 pb fragment located upstream from the ATG site of the coding sequence identified SEQ ID NO: 4 and corresponds to a fragment encompassing nucleotide 464 to nucleotide 1797 of the sequence identified under SEQ ID NO: 3.

[0011] Some results have been disclosed in WO 97/06259 showing that plants susceptible to wilt inducing fungi of *Fusarium* species, especially *Fusarium oxysporum f.sp. lycopersici* race 2, are rendered resistant to said *Fusarium* when the coding sequence of the I-2 genes is transferred in said plants under the control of the above 1.3 kb fragment.

[0012] In order to examine the conditions and level of transcription of the I-2 resistance gene when placed under the control of a DNA sequence encompassing the above-said 1.3 kb fragment, and especially in view of the experimental results obtained that show a low abundance of transcripts of the I-2 gene in some plant tissues, for instance in root, stem and leaf tissues of recombinant plants, the inventors have decided to further investigate the functionality of the I-2 5' coding sequences showing a regulatory activity on the transcription of the I-2 gene, and have therefore designed and tested recombinant constructs using a reporter gene placed under the control of nucleotide sequences comprising a regulatory sequence active on regulation of the transcription of the I-2 gene.

[0013] From the results obtained with DNA constructs using the regulatory transcription sequences of the I-2 gene, further compared with results obtained with the same reporter gene placed under the control of a different plant specific promoter, the inventors have characterized specific properties of the sequences disclosed in the present patent application, having a regulatory transcription activity: these properties include ability to enable a tissue specific expression of determined sequences placed under the control of these regulatory sequences.

[0014] Therefore, the invention provides means which can be used for regulation of the tissue specific expression in plant cells or plants, of nucleic acid sequences, especially as a result of regulation of the transcription of said nucleic acid sequences.

[0015] The invention therefore relates to a nucleotide sequence having a regulatory activity on the transcription of the I-2 resistance gene in determined plant host cells, which nucleotide sequence comprises a DNA sequence of around 3.8 kb derived from the 5' non coding region of the I-2 gene. Said sequence has preferably a size in the range of 5 to 1 kb.

[0016] According to a preferred embodiment, said regulatory sequence comprises the sequence depicted as SEQ ID NO:1, corresponding to a 3.8 kb fragment that can be obtained or derived from the 5' non coding region located upstream from the translation initiation codon (ATG) of said I-2 resistance gene. The coding sequence of the I-2 gene is represented as SEQ ID NO: 4.

[0017] The invention relates especially to said 3.8 kb fragment which can be obtained from the B22 cosmid and corresponds to an EcoRI/EcoNI fragment of 3.8 kb of the 5' end of the I-2 gene.

[0018] The invention especially relates to a nucleotide sequence according to the above definition, which sequence is SEQ ID NO: 1.

[0019] The above-identified sequence SEQ ID NO: 1 comprises a fragment located 5' from the ATG codon of the I-2 resistance gene which is sufficient for the transcription of said gene.

[0020] The invention also relates to the use of the 1.3 kb fragment derived from the 5' non coding region of the I-2 gene, said sequence being disclosed as SEQ ID NO:2 and containing nucleotide 464 to 1797 of the I-2 gene. It also concerns the use of the sequence designated SEQ ID NO:3 corresponding to nucleotides 1 to 1798 upstream from the ATG codon in the I-2 gene.

[0021] According to a particular embodiment, the invention also relates to DNA sequences retaining a regulatory activity of the sequences described above, especially of SEQ ID NO: 1, SEQ ID NO:2 or SEQ ID NO:3 which sequences are derived from the above by deletion, insertion, substitution of one or several nucleotide residues.

[0022] Despite their ability to regulate transcription of nucleic acid sequences, the choice of a specific sequence among the preceding one can lead to a modification of the level of the transcription.

[0023] Among derived regulatory nucleotide sequences used in accordance with the invention, those hybridizing under stringent conditions with one of the above described sequences are of interest to achieve the invention. Advantageously, said derived sequences have substantially the same size or a shorter size with respect to the sequence from which they are derived.

[0024] Stringent conditions refer to hybridization conditions which allow a nucleic acid sequence to hybridize to a particular sequence. In general, high stringent conditions refer to the hybridization conditions which allow a nucleic acid sequence of at least 50 nucleotides and preferably about 200 or more nucleotides to hybridize to a particular sequence at about 65 °C in a solution comprising about 1 M salt, preferably 6 x SSC or any other solution having a comparable ionic strength, and washing at 65 °C in a solution comprising about 0,1 M salt, or less, preferably 0,2 x SSC or any other solution having a comparable ionic strength. These conditions allow the detection of sequences having about 90 % or more sequence identity. In general, lower stringent conditions refer to the hybridization conditions which allow a nucleic acid sequence of at least 50 nucleotides and preferably about 200 or more nucleotides to hybridize to a particular sequence at about 45 °C in a solution comprising about 1 M salt, preferably 6 x SSC or any other solution having a comparable ionic strength, and washing at room temperature in a solution comprising about 1 M salt, preferably

6 x SSC or any other solution having a comparable ionic strength. These conditions allow the detection of sequences having up to 50 % sequence identity. The person skilled in the art will be able to modify these hybridization conditions in order to identify sequences varying in identity between 50 % and 90 %.

[0025] Alternatively, stringent conditions refer to hybridization conditions which allow a nucleic acid sequence to hybridize selectively to the *I-2* resistance gene in its genomic environment, substantially to the exclusion of hybridization with other DNA sequences of said genomic environment.

[0026] According to a preferred embodiment, the invention concerns regulatory nucleotide sequences according to the above definition or a fragment of said sequences which are capable of driving a tissue specific transcription of a determined nucleic acid sequence placed under their control.

[0027] By the expression "driving a tissue specific transcription", it is encompassed any sequence which would impact on the regulation of said transcription in a host cell and especially in a tissue specific manner, especially those capable of initiating said transcription.

[0028] The invention also relates to a recombinant nucleotide sequence comprising one of the above defined regulatory nucleotide sequence, which is functionally linked to a determined nucleic acid sequence of interest.

[0029] By the expression "functionally linked", it is meant that when the regulatory nucleotide sequence is used in combination, for instance in a fusion, with the nucleic acid sequence of interest, and when possible additional elements involved in the regulatory activity are present or activated, a regulatory activity enabling the transcription of said nucleic acid interest is obtainable in competent host cells.

[0030] According to a particular embodiment, the invention relates to a recombinant nucleotide sequence, wherein the regulatory nucleotide sequence is functionally linked to the nucleic acid of interest and wherein this construct further comprises 3' from the nucleic acid of interest, a non translated sequence comprising a transcription termination sequence. This transcription termination sequence can especially contain polyadenylation sites.

[0031] The nucleic acid sequence of interest can be of various type or origin especially originating from plants, animals, bacterial, viral organisms. It is especially the sequence encoding the *I-2* resistance gene product.

[0032] Alternatively, said nucleic acid sequence of interest can be chosen among sequences having a direct or indirect activity in resistance against pathogens, especially resistance against plant pathogens, particularly those having a targeted tissue specificity. As example, resistance can be mediated through an interaction with the site of penetration of the pathogen, or of the infection capacity of the pathogen or through an effect on the colonization of tissues in infected plants.

[0033] In accordance with the invention, the nucleic acid sequence of interest can be chosen among sequences which are naturally expressed in a tissue specific manner in their natural host, or for which no tissue specific expression exists in the natural host. In this latter case especially, a tissue specific expression would be specifically obtained by the use of the regulatory sequence of the invention when it is combined to the chosen sequence, said chimeric construct being then transferred into a host plant or plant host cell.

[0034] The invention is especially directed to the use of the recombinant nucleotide sequence defined above for a specific expression in a plant vascular tissue. According to the invention, the expression "plant vascular tissue" encompasses tissues having vascular cells whatever their location in the plant. Vascular tissues are especially present in roots, stems or leaves. The expression therefore encompasses cells, or cell layers from vascular origin or having a vascular related function. It comprises cell layer(s) surrounding the lateral root primordia or cell layers at the base of adventitious roots, or cells in the leaf veins, in the plant fruits or vegetable, or in the stigma of the style.

[0035] The inventors have shown that the use of the regulatory sequences of the invention may be of specific interest for the expression of nucleic acid sequences in plant lateral root meristem and tissues deriving from said meristem.

[0036] Among sequences which are regarded as sequences of interest to be expressed in a tissue specific manner within host plants, genes or generally DNA constructs having activity in mediation of resistance to a plant pathogen are preferred.

[0037] Among these plant pathogens, those qualifying as root pathogens and accordingly invading plants through the roots, are of interest. As an example, fungi, nematodes are of interest and especially fungi of *Fusarium* species are cited.

[0038] The recombinant nucleotide sequences of the invention can be used for transfer in plants or in plant cells, according to any available technique. Particularly recourse may be made to the use of vectors especially plasmids or cosmids, containing said recombinant nucleotide sequence for transforming plant cells or plants.

[0039] The invention therefore is directed to recombinant cells comprising a nucleotide sequence defined above, including recombinant nucleotide sequences according to the invention, or vectors containing the same.

[0040] Among appropriate plant cells which can be chosen as targets for the expression of the nucleic acid of interest placed under the control of the regulatory sequences of the invention, bacterial or eukaryotic cells can be cited.

[0041] Among eukaryotic cells, most preferred cells are plant cells and especially plant cells originating from vascular tissues.

[0042] According to another embodiment of the invention, said plant cells are root cells, stem cells or leaf cells.

[0043] The invention also relates to plants which are recombined with the nucleotide sequences which have been disclosed above.

[0044] According to a specific embodiment of the invention, said recombinant plants are capable of expressing a gene having a mediation activity of resistance to a plant pathogen. Particularly, plant pathogens among those cited above are preferred and especially when they are known to have a tissue specific invading activity.

[0045] Recombinant plants according to the invention can be derived from recombination with the nucleotide sequences of the invention of tomato, egg plants, potato, melon, tobacco or Arabidopsis.

[0046] The invention is also directed to a process for obtaining plants having reduced susceptibility to a plant pathogen, comprising the following steps:

- i) inserting into the genome of a plant cell a nucleotide sequence defined above, or a vector defined above,
- ii) obtaining transformed plant cells,
- iii) regenerating from said transformed plant cells genetically transformed plants, and
- iv) optionally, propagating said plants.

[0047] The above defined process is of specific interest to obtain transgenic plants which are further selected for having a reduced susceptibility to a plant pathogen as a result of the expression of the nucleic acid of interest under the control of the regulatory sequences of the invention.

[0048] The invention is also directed to a process for protecting plants in cultivation against plant pathogen infection, which comprises:

- i) providing the genome of plants with a nucleotide sequence defined above, or a vector defined above,
- ii) growing said plants.

[0049] Further details and advantages regarding the invention appear from the following examples and figures.

[0050] **Fig. 1.** Transcripts of *I-2* and *I-2* homologs detected by RT-PCR. **A.** Schematic representation of the *I-2* region used for cDNA amplification. Primers are indicated by arrows, gray boxes are the region of variable repeats between *I-2* and *I-2* homologs. **B.** The by RT-PCR amplified fragments. KG52201 is Fusarium susceptible line; C295 is *I-2* containing Fusarium resistant line; M=marker (kb); D=DNA; r=RNA from root; s=RNA from stem; l=RNA from leaf; arrow indicates expected length *I-2* transcript.

[0051] **Fig. 2.** Comparison of GUS activity in seedlings of transgenic tomato plants of line KG52201 and C295. Each of the 15 squares per line represents the activity of 5 pooled nonselected seedlings of 15 individual transgenic lines. The mean and standard deviation of the 15 samples are presented besides. Arrows points out lines used for further analysis.

[0052] **Fig. 3A.** Comparison of GUS activity in roots, stems and leaves of transgenic lines. Bars represent the expression of material pooled from 10 selected transgenic plants.

[0053] **Fig. 3B.**

A. seedlings 4, 5 and 7 days-old

B. tomato fruit (not ripe)

V= vascular bundle

C. seed

H=hypocotyle, C=cotyledons

D. stem

AR=adventitious root (premordia), Fr=fibrous root, Rt=root tip

E. vascular bundle of stem

Co=cortex, E=endodermis, Pi=pericycle, Ca=cambium, Ph=phleom, Xp=xylem parenchyma, X=xylem mature

F. central cylinder of root

Co=cortex, E=endodermis, Ca=cambium, Ph=phleom, Xp=xylem parenchyma, X=xylem mature, Px=premature xylem

G. lateral root formation

Lr=lateral root, V=vascular tissue, Rh=root hairs

H. leaf, I-2 expression upper leaf, under 35SCaMV leaf

Ve=veins

[0054] **Fig. 4.** Comparison of GUS activity in stem of Fusarium infected and water treated transgenic plants. Bars represent the expression of 20 pooled stems of nonselected transgenic plants.

[0055] **Fig. 5.** Macroscopical localization of *uidA* gene expression under control of the 5' flanking region of the *I-2*

gene. **A**, Seed. **B**, Germinated seed, right *I-2* promoter, left 35SCaMV promoter. **C**, 7-days-old seedling. **D**, Lateral root primordia, top view. **E**, Lateral root primordia, side view. **F**, Base of lateral root, side view. **G**, Mature tap root. **H**, Stem. **I**, Adventitious root formation. **J**, Leaf, upper *I-2* promoter, bottom 35SCaMV promoter. **K**, Tomato fruit. **L**, Flower. c = cotyledons, h = hypocotyl, lrp = lateral root primordia, r = root, arp = adventitious root primordia, vb = vascular bundle, st = stigma, s = style, o = ovary.

[0056] **Fig. 6.** Histochemical localization of *uidA* gene expression under control of the 5' flanking region of the *I-2* gene. **A**, Young root. **B**, Vascular tissue of young root. **C**, Mature tap root. **D**, Vascular tissue of tap root. **E**, Stem section. **F**, Detail of stem. **G**, Detail of vascular bundle of stem. **H**, Stem section of three-weeks-old plant. vt = vascular tissue, ca = cambial zone, co = cortex, xp = xylem parenchyma, x = xylem, px = premature xylem, e = endodermis, p = phloem, ip = inner phloem, r = rays.

[0057] **Fig. 7.** Histochemical localization of *F. oxysporum* f.sp. *lycopersici* infection by using constitutive expressing *uidA* transgenic *Fusarium* isolate, seven days after inoculation. **A**, Infected root of susceptible plant. **B**, Infected root of resistant plant. **C**, Detail of infected root of susceptible plant. **D**, Detail of infected root of resistant plant. **E**, Stem section of susceptible plant. **F**, Stem section of resistant plant. co = cortex, vt = vascular tissue, f = *Fusarium*, ca = cambial zone, x = xylem, xp = xylem parenchyma.

[0058] **Fig. 8.** Constructs

A: Steps for the construction of pJM2005

B: pJM2005 containing an *I-2* promoter *uidA* chimeric gene with long *I-2* promoter sequence (3.8 kb)

[0059] **Fig. 9.** Sequence of the 3.8 kb DNA non coding fragment.

Expression of the *Fusarium I-2* resistance gene co-localizes with the site of defence response

[0060] The expression of the *Fusarium* resistance gene *I-2* of tomato has been analysed. Although *I-2* transcripts were undetectable by Northern blot analysis, RT-PCR revealed that *I-2* and at least five *I-2* homologs are expressed in roots, stems and leaves of young tomato plants. Plants transformed with chimeric constructs containing a functional *I-2* promoter fused to the β -glucuronidase (GUS) reporter gene were used in detailed expression studies. GUS-activity was found in unchallenged plants; infection with *F. oxysporum* f.sp. *lycopersici* race 2 did not alter promoter activity. Macroscopical analysis showed that the *I-2* promoter drives expression of the reporter gene in a cell layer beneath the lateral root primordia, in mature roots, at the base of adventitious roots and in vascular tissue of stems, leaves and fruits. The vascular specific expression was confirmed by microscopical analysis, which revealed expression in endodermis, cambial zone, phloem, xylem parenchyma cells, premature xylem vessels and rays cells. We show that in resistant plants fungal growth into this region of the vascular tissue is prevented suggesting a direct role of *I-2* in mediation the resistance response of tomato to *F. oxysporum* f.sp. *lycopersici*.

[0061] Tomato may become infected by the soil-borne pathogen *Fusarium oxysporum* f.sp. *lycopersici*. The fungus enters the plant via the roots either by active penetration at the root tip (Bishop and Cooper, 1983) or via natural wounds, for instance via cortex tissue that is disrupted by the formation of lateral roots (Hutson and Smith, 1983). Penetration of vascular elements depends on the presence of cells that are injured, senescent or necrotic, or circumstances providing entry through wounds (Beckman, 1987). In xylem vessels the fungus spread by long distance transport using the transpiration stream (xylem flow). Fungal spores germinate within the vascular elements, grow towards pits and colonize adjacent xylem parenchyma cells. Colonization of secondary xylem parenchyma cells has been shown to be typical for susceptible plants (Beckman et al., 1989). These living xylem-contact-cells are held responsible for defence responses in lateral and longitudinal directions, including callose deposition, production of secondary metabolites, lignification and vascular occlusion by gels, gums or tyloses (Beckman, 1987; Beckman and Roberts, 1995). Defence responses of susceptible and resistant plants are basically the same, only the timing and strength of the response is different (Beckman, 1987).

[0062] The dominant resistance gene *I-2* of tomato confers resistance to *F. oxysporum* f.sp. *lycopersici* race 2. An AFLP-based positional cloning strategy was used to clone the *I-2* gene which is located within a cluster of seven homologs (Simons et al., 1998). The *I-2* gene encodes a protein with similarities to a large family of *R* gene products containing a nucleotide binding site and a leucine-rich repeat (NBS-LRR) (Simons et al., 1998; Hammond-Kosack and Jones, 1997; Ellis and Jones, 1998). The structural domains predict a role in signalling, transducing a signal from the invading pathogen to the activation of defence related genes (Hammond-Kosack and Jones, 1997; Ellis and Jones, 1998).

[0063] The characterization of *I-2* promoter activity could help to localize the actual site of the resistance reaction. How resistance genes function and whether these genes encode other functions in plants apart from mediation of resistance, is not known. Gene expression analysis could lead to a better understanding of the resistance mechanism as well as to suggestions for other functions of *R* genes. For none of the cloned resistance genes such characterisations

have been published until now. To analyse *I-2* promoter activity, tomato plants were transformed with chimeric constructs containing a functional *I-2* promoter region fused to the β -glucuronidase (GUS) reporter gene. Macroscopical and histochemical analysis revealed a specific expression in cells neighbouring the xylem vessels, suggesting a very specifically localized defence reaction against *F. oxysporum* f.sp. *lycopersici*.

MATERIAL AND METHODS

[0064] **Bacterial and fungal strains.** *E. coli* strain DH5a was used for plasmid propagation. Infection test were performed using *F. oxysporum* f.sp. *lycopersici* race 2 isolate Fol007.

[0065] **Plant material.** Tomato line KG52201 was used as susceptible line containing no resistance to any race of *F. oxysporum* f.sp. *lycopersici*. Line OT105 (E22) was used as a control line because the *I-2* resistance gene was cloned from this line (Simons et al., 1998). OT105 is homozygous, and is resistant to *F. oxysporum* f.sp. *lycopersici* race 2 and susceptible to race 1. Moneymaker line C295 is resistant to *F. oxysporum* f.sp. *lycopersici* race 1 and race 2.

[0066] For RNA isolation plant tissue was grounded in liquid nitrogen. After addition of extraction buffer (100 mM Tris-HCl pH 8.5, 100 mM NaCl, 20 mM EDTA and 1% sarkosyl) RNA was extracted with phenol and twice with phenol/chloroform (1:1) before it was precipitated with 2-propanol. DNA/RNA pellet was dissolved in water and RNA was precipitated by addition of an equal volume of 4M LiCl. The RNA pellet was dissolved in H₂O, precipitated with ethanol and redissolved in 20-100 μ l H₂O. Ten μ g of total RNA was separated on 1.5% denaturing formaldehyde agarose gel electrophoresis and subsequently blotted on Hybond-N membrane (Amersham). The *I-2* probe was ³²P-labelled by the Random Primers Labelling System (BRL Life technologies Inc., USA) and hybridization was performed in 0.5M phosphate buffer pH 7.2 containing 7% SDS, 1% BSA and 1mM EDTA. After overnight incubation at 65°C, the blot was washed with 2x and 1x SSC, respectively, for 15 min each.

[0067] For cDNA synthesis 50 ng oligo dT primer was added to 5 μ g of RNA in a volume of 6.2 μ l. After annealing, 3.8 μ l was added containing 0.4 μ l dNTP 10 mM, 1 μ l DTT 100 mM, 2 μ l 5x RT buffer, 0.2 μ l RNase inhibitor (30U/ μ l) and 0.2 μ l Superscript II (200U/ μ l) (Gibco-BRL). The mixture was incubated at 45°C for one hour. PCR was performed under standard conditions using 2 μ l of cDNA. Primers 3449 (5' CCTCCTTTTCTCACCTCACTCGC 3') and PF95 (5' GTACCAGACTTGTCGTACTCGCTC 3') were used to amplify the 3' end of both the active *I-2* gene and other *I-2* homologs.

[0068] **Constructs.** To construct an *I-2* promoter *uidA* chimeric gene, an *NcoI* restriction site was created at the translation start of *I-2*. This resulted in a change of two bases upstream of the translation initiation codon from AA to CC. The *NcoI* site was introduced by PCR using primer FP4 (5' CTGCTAAGCTTATCTCCATGGCTCAAATC 3') together with primer FP3 (5' GTTGTTTGATATCTTATCAG 3') spanning an *EcoRV* restriction site located 806 bp upstream of the ATG. The fragment was cloned in pBluescript which then was called pJM1001 (see figure) and sequencing confirmed that it was identical to the original sequence of the 5' flanking region of *I-2*. An *EcoRI/NcoI* fragment, containing the 806 bp PCR *I-2* promoter sequence of pJM1001 was used to exchange the *EcoRI/NcoI* 35S promoter fragment of pMOG901 (ZENECA-MOGEN, Leiden, the Netherlands). This resulted in plasmid pJM1002 containing the 806bp *I-2* promoter fragment fused to the β -glucuronidase gene (*uidA* with modified intron) with the *nos* terminator region. To introduce more cloning sites the 805bp *I-2* promoter-GUS fragment was *EcoRI/HindIII* cloned in pBluescript KS+ (pJM1003). An *EcoRI/EcoNI* fragment of 3.7 kb of the 5' flanking region of *I-2* was used, in a two step cloning, to partial exchange the PCR based 806 bp sequence (105 bp PCR fragment remained but which was identical to the original sequence) and extend the promoter sequence. This resulted in pJM1005 containing 3.8 kb 5' flanking region of the *I-2* gene fused to *uidA* gene and the *nos* terminator. This construction enables to make a restriction site at the ATG codon therefore enabling to make constructs which start directly at the ATG codon.

[0069] Using *EcoRI* and *XhoI* the 3.8 kb *I-2* promoter-GUS fragment was cloned into the binary vector pMOG402 (ZENECA-MOGEN, Leiden, The Netherlands) containing the Left and right border, PPTII regulated by *nos* 5' and 3' sequences. This plasmid was called pJM2005.

[0070] Using the same strategy, an *NcoI* site was introduced at the ATG of the *I-2* coding region using primers FP70 (5' CAGATTTGAGCCATGGAGATTG 3') and FP71 (5' GCTGACCTTCCACCTTAAG 3'). The first primer introduces the *NcoI* site, the second is spanning a *SacI* restriction site for further cloning strategies. The amplified fragment was cloned, sequenced and used to exchange the *I-2* sequence in pKG 6016.

[0071] In a three point ligation the *EcoRI/NcoI* promoter fragment of pJM1005 and the *NcoI/XhoI* 3801 bp coding region of *I-2* and 1100 bp *I-2* downstream sequence were cloned into the *EcoRI/XhoI* digested pMOG402, resulting in pMH4002.

[0072] **Plant transformation.** The binary T-DNA vectors pJM2005 and pMH4002 were introduced into *Agrobacterium tumefaciens* strain EHA105 by electroporation. Constructs were checked for recombination events by transformation of *E. coli* DH5 α with plasmid isolated from transformed *A. tumefaciens* followed by restriction analysis. Tomato cotyledons were transformed essentially as described by Fillatti et al. (1987). Ploidy level of transformants was checked by counting the number of chloroplasts in the stomatal guard cells.

[0073] **Infection assay.** Infection assays were performed as described previously (WO 97/06259).

[0074] **Quantitative GUS assay.** GUS activity was quantified fluorometrically by using the substrate 4-Methylumbelliferone glucuronide (MUG) as described by Jefferson (1987). Reactions were performed in 50 µl, containing 1 mM MUG and stopped after 2 hours incubation at 37°C by addition of 0.5 M Na₂CO₃. The fluorescence was related to a calibration curve of 4-methylumbelliferone (MU). Protein determination was carried out using Bradford reagents and BSA as standard.

[0075] **Histological observation.** GUS assays were performed histochemically with 5-bromo-4-chloro-3-indoyl glucuronide (X-Gluc) according to Jefferson (1987) and modified by Toriyama et al. (1991). Plant tissues were emerged in the GUS staining solution (1 - 0.5 mM X-Gluc, 0.1 M sodium phosphate buffer, pH 7.0, 0.5% Triton X-100, 2 mM ferrocyanide and 2 mM ferricyanide) and vacuum infiltrated for at least one hour in total. Incubations were at 37°C overnight after which plant material was fixed in FPA (Formalin 2%, Propiono acid 5% in ethyl-Alcohol 63%) and subsequently incubated in 70 % ethanol. Root and stem sections were embedded in paraffin and sectioned at 120 µm using a sledge microtome.

RESULTS

Expression of the *I-2* gene family

[0076] Northern blot analysis on total RNA from roots, stems and leaves of *I-2* containing tomato (line C295) did not give any hybridization signal using *I-2* DNA as probe. RNA isolated from *Fusarium*-infected plants did not yield a signal either. These results suggest that the transcription level of *I-2* and other members of the *I-2* family is very low. Alternatively, *I-2* transcripts may be very unstable. In a next attempt to detect *I-2* transcripts an RT-PCR approach was followed. Homologs of *I-2* differ from the active gene by the number of a completely conserved 23 amino acid repeat (Simons et al., 1998). Within *I-2* three tandemly arranged copies of this repeat are present, whereas in homologs the number of copies vary from two to six. A set of two primers was designed spanning a sequence containing the region with this variable number of repeats and the intron in the 3' UTR (Fig. 1A). Using this primer set fragments amplified on genomic DNA can be distinguished from fragments amplified on cDNA, and the *I-2* transcript can be distinguished from transcripts of *I-2* homologs (Fig. 1A). On genomic DNA as template the presence of at least three homologs could be demonstrated in susceptible line KG52201 and at least five homologs in the resistant line C295 (Fig. 1 B, lanes D). As expected, fragments amplified on cDNA are smaller due to the absence of the intron. RNA isolated from roots (r), stems (s) or leaves (l) of young plants yielded at least six transcripts in the *I-2* containing line C295 and three *I-2*-like transcripts in susceptible line KG52201. The expected size of the *I-2* fragment is indicated by an arrow. This result shows the presence of *I-2* transcripts in root, stem and leaf in the absence of a *Fusarium* infection. The low abundance of transcripts and the expression of various homologs with indistinguishable 5' and 3' ends prompted us to use *I-2* promoter-GUS fusions to study the expression of *I-2*.

Functional expression of *I-2* in transgenic plants

[0077] To investigate the functionality of the *I-2* promoter fragment to be used in expression studies, a construct was made consisting of a 3.8 kb 5' flanking sequence, the *I-2* coding region and a 1.1 kb 3' flanking sequence. This construct was used to transform the race 1 and 2 susceptible tomato line KG52201. Seventeen individual transformants were selected and selfed. R1 lines were tested for resistance to *F. oxysporum* f.sp. *lycopersici* race 2 in standard root dip inoculation experiments. To this end 20 plants of each line segregating for the transgene were tested. Plants were inoculated and potted separately to enable a reliable quantification of resistance by measuring the weight of plants three weeks after infection (WO 97/06259). Table 1 shows the results of a representative experiment. Control lines show susceptibility (KG52201) or resistance (OT105 and C295) as expected. Two (KG.14 and KG.05) out of seven transgenic lines tested were found to be resistant to *F. oxysporum* f.sp. *lycopersici* race 2: after infection their mean weights are significantly ($p=0.001$) different from the susceptible control and are almost as high as the water control. KG.14 and KG.05 showed the expected 3:1 (resistant : susceptible) segregation of the resistance trait. The other five lines containing the same construct either did not differ significantly from the susceptible control line (KG.15) or showed such a reduction in plant weight compared to the water control that they all must be considered susceptible (Table 1). In total five lines (29%) of the 17 lines tested were found to be resistant. From this we conclude that for functional expression of the *I-2* resistance gene the 3.8 kb promoter fragment is sufficient.

Table 1.

| Resistance of transgenic lines complemented with the <i>I-2</i> gene flanked by 3.8 kb 5' flanking region and 1.1 kb 3' downstream region. | | | | |
|--|--------------------------|------------------------------|----|-------------------|
| Tomato line | Water treated weight (g) | Fusarium infected weight (g) | | Segregation (R:S) |
| KG52201 | 15.8±1.5 | 0.8±1.6 | a | 0 : 20 |
| KG.15 | 12.5±2.1 | 2.4±2.9 | ab | 0 : 18 |
| KG.13 | 12.0±1.2 | 2.6±3.0 | b | 1 : 19 |
| KG.16 | 12.7±1.4 | 3.4±2.8 | b | 0 : 20 |
| KG.18 | 13.2±1.6 | 3.5±3.3 | b | 2 : 18 |
| KG.02 | 11.7±1.6 | 3.7±3.1 | b | 1 : 19 |
| | ± | ± | | |
| KG.14 | 12.7±2.6 | 9.1±3.7 | c | 14 : 5 |
| KG.05 | 12.0±0.5 | 10.0±4.1 | cd | 15 : 3 |
| OT105 | 17.1±1.5 | 11.8±1.5 | d | 20 : 0 |
| C295 | 18.0±1.6 | 14.3±1.1 | e | 19 : 0 |

I-2 promoter driven expression of the GUS reporter gene

[0078] Transgenic lines containing the 3.8 kb 5' flanking sequence of the *I-2* gene fused to the *uidA* (GUS) reporter gene were analysed for expression. R1 seedlings of 15 individual transgenic lines of both KG52201 and C295 were grown in potting soil. Five plants per line were pooled and tested for expression using the quantitative MUG assay. Between transgenic lines expression levels appeared to be highly variable (Fig. 2). Mean expression levels were higher in C295 transgenic than in KG52201 lines, although the differences were not found to be significant ($p=0.065$). Of each cultivar, four transgenic lines showing different levels of expression were selected for further analysis. Roots, stems and leaves of kanamycin resistant seedlings were pooled and tested separately. Of each transgenic line material of ten plants was pooled. The variation in GUS activity (Fig. 3) between the different transgenic lines correlated with the results obtained before (Fig. 2). In all lines GUS activity was found to be highest in stems and lowest in leaves.

Histochemical analysis of tissue specific expression of the *I-2* promoter in unchallenged plants

[0079] Eight lines transgenic for *I-2-gus-nos* of both cultivars KG52201 and C295 were selected for detailed histochemical analysis. These transgenic plants all expressed the transgene in an identical pattern, although expression levels seem to vary. In most cases a lower intensity of blue staining was found in the transgenic lines of KG52201.

[0080] Seeds and seedlings were analysed for GUS activity. The seed embryo showed GUS expression in hypocotyl and cotyledons. The same was found when the seeds had germinated into young seedlings, but no expression was detectable in the roots on microscopical level. In a later stage, young roots showed a very faint staining in some of the scarce xylem parenchyma cells. In these young roots, a very pronounced expression was found at the origin and base of lateral root formation. In an early state of lateral root primordia, GUS staining was visible as a circle. When lateral root primordia further developed in a lateral root, expression remained visible at the root base where it has escaped from the older root. The signal becomes more diffuse during growth of the root, probably by dilution of the initial expression because of cell elongation. When secondary thickening of the root has occurred resulting from cambial activity, high GUS activity could be detected. Low activity was found in the endodermis, phloem and vascular cambium. A very pronounced activity could be observed in xylem parenchyma cells surrounding the xylem vessels as well as in premature xylem vessels where cytoplasm was still present. In primary meristem of the root tips no expression was found.

[0081] Expression in stem was detected both in young and old plants. The GUS activity was localized in the same tissue as described for the root: endodermis, phloem and cambial tissue xylem parenchyma cells and xylem vessels when still containing cytoplasm.

[0082] Secondary lateral growth of the stem resulted in more cambial tissue and xylem vessels, both showing pronounced GUS staining. Especially, the ray parenchyma cells, the living cells that are located adjacent to the secondary xylem show a pronounced GUS activity. Analogue to the expression of GUS by the *I-2* promoter at the base of lateral root formation, expression was abundantly present at the base of adventitious roots which originate from the stem.

[0083] The *I-2* promoter also drives GUS expression at the leaf veins, likely with the same tissue specificity as described for root and stem. That the staining is not due to uncompleted substrate infiltration is shown by the comparison

with a 35SCaMV transgenic leaf which revealed GUS activity in the complete leaf. The vascular specific expression of I-2 is also found in the tomato fruit. Staining can be observed in the whole fruit but is most abundantly present in the vascular veins. Surprisingly, the I-2 promoter is also active in the stigma of the style.

| | I-2 promoter | 35SCaMV |
|--|--------------|---------|
| seed hypocotyl | + | + |
| young (fibrious) root | - | + |
| tap root and secondary thickened roots | + | + |
| adventive root primordia base | + | - |
| adventive root (fibrious root) | - | + |
| adventitious root base | + | - |
| young stem | + | + |
| older stem | + | + |
| exodermis | - | + |
| cortex | - | + |
| endodermis | + | + |
| pericycle | - | + |
| cambium | + | + |
| phloem (internal and secondary) | + | + |
| xylem parenchyma | + | + |
| xylem (premature) | + | ? |
| ray parenchyma cells (pith rays) | + | + |
| hairs | - | + |
| leaf mesophyll | - | + |
| leaf vein | + | + |
| gladular trichomes | + | - |
| stigma of pistil | + | + |
| tomato fruit | + | + |

[0084] To examine induction of expression by *Fusarium*, the same eight lines were used. Of each line 20 R1 seedlings that had not been selected for the presence of the transgene, were infected with *F. oxysporum* f.sp. *lycopersici* race 2 by a standard root dip method or were mock inoculated with water. Seven days after infection, when susceptible plants started to show *Fusarium* wilt symptoms, stems of the seedlings were analysed for GUS activity. No induction nor suppression of GUS activity was found in inoculated plants (Fig. 4). From these results we conclude that I-2 promoter driven expression is not changed by *Fusarium* infection. However, we cannot yet exclude changes in expression levels in tissue around the site of colonization since such changes can not be detected with this type of experiments.

Macroscopical analysis of GUS expression

[0085] The transgenic lines used before of both cultivar KG52201 and C295 were used for analysis of the tissue specificity of expression. In all cases expression patterns were identical, although levels varied. Since C295 lines showed most pronounced GUS staining, these lines were used in further studies. The seed embryo displayed GUS activity in hypocotyl and cotyledons (Fig. 5A). The same was found when seeds had germinated (Fig. 5B and C). At macroscopical level, no expression was visible in the roots. For comparison CaMV 35S promoter driven GUS expression in roots is shown (Fig. 5B, right-hand seedling). At sites where tap roots developed lateral roots, a very pronounced expression was found in a cell layer beneath the lateral root primordia (Fig. 5D; E and F). In an early stage of lateral root formation, GUS staining was visible as a circle when viewed from above (Fig. 5D). A side view showed the expression in a single layer of cells beneath the lateral root primordia (Fig. 5E). When these primordia further developed into lateral roots, expression remained visible, in particular at the root base where it had emerged from the older root (Fig. 5F). The signal became more diffuse during growth of the root probably because of cell elongation. GUS activity could be detected in mature roots that had grown by secondary thickening (Fig. 5G). In the primary meristem of root

tips no expression was found. Expression in the hypocotyl was visible both in young and old plants. GUS activity was localized in the central part of the stem, most likely in the vascular tissue (Fig. 5H). In analogy with expression of the GUS gene at the base of lateral root primordia, expression was also found at the base of adventitious roots originating from the stem (Fig. 5I). The *I-2* promoter drives GUS expression in leaf veins as well (Fig. 5J, upper leaf), again demonstrating vascular specificity. For comparison 35S promoter driven GUS expression in the leaf is shown as well (Fig. 5J, bottom leaf). Vascular specific expression is also found in tomato fruit where staining is most abundantly in the vascular bundles (Fig. 5K). Finally, the *I-2* promoter is active in the stigma of the style (Fig. 5L).

Histochemical analysis of expression of the GUS reporter gene

[0086] In young roots low GUS activity was found in the few xylem parenchyma cells and premature xylem vessels (Fig. 6A and B). In older roots in which xylem tissue has expanded because of cambial activity, primarily vascular tissue was stained (Fig. 6C and D). Detailed analysis revealed expression in the cambial zone, xylem parenchyma cells and premature xylem vessels where cytoplasm was still present (Fig. 6D). In stems, where the central xylem developed into separate vascular bundles, GUS activity was localized not only in the same tissues as described for the root: cambium, xylem parenchyma cells and premature xylem vessels (Fig. 6E and F), but also in the endodermis, the phloem and inner phloem vessels. Remarkably GUS activity is highest in premature xylem vessels in all tissues analyzed (Fig. 6G). Secondary lateral growth of the stem results in more cambial tissue, xylem parenchyma cells and xylem vessels (Fig. 6H). Especially the cambial zone and the rays, the living cells that are located adjacent to the secondary xylem, show a pronounced GUS activity.

Histochemical localisation of *Fusarium* in infected tomato.

[0087] Detailed studies of *F. oxysporum* f.sp. *lycopersici* colonizing tomato have been published (Beckman 1987; Beckman and Roberts 1995). However, to be able to correlate the *I-2* promoter activity in the same experimental design as presented in the previous section, we analyzed the colonization of tomato by a GUS marked *Fusarium*. Non-transgenic KG52201 (susceptible) and C295 (resistant) plants were inoculated with a transgenic *F. oxysporum* f.sp. *ycopersici* race 2 isolate expressing the GUS gene driven by the constitutive glyceraldehyde-3-phosphate dehydrogenase promoter (Roberts et al., 1989). Three, five and seven days after inoculation, plants were harvested for detailed histochemical analysis. Roots of both susceptible and resistant plants were colonized by *F. oxysporum* f.sp. *lycopersici* within three days. Five days after inoculation, roots and hypocotyl of all susceptible plants were colonized and colonization went on until day seven. Three days after inoculation the colonization was also visible in xylem vessels and expanded to xylem parenchyma cells of the roots, and often even further into the cambial zone at days five and seven (Fig. 7A and C). In resistant plants roots showed a much lower level of GUS activity and in only a few cases (10% of the plants observed) localized GUS staining was found higher up in the tap root or in the stem. Microscopical analysis showed that the fungus was restricted to the xylem vessels and occasionally to a single layer of xylem parenchyma cells (Fig. 7B and D). Around the infected xylem bundle a brownish discoloration was found, probably of phenolic origin (Fig. 7D and F). In the stem of susceptible plants, *Fusarium* colonization spreaded from out the xylem vessels into xylem parenchyma cells and cambial cells (Fig. 7E), whereas in resistant plants *Fusarium* was restricted to the xylem vessels and some xylem parenchyma cells (Fig. 7F). These results indicated that there is a co-localization of *I-2* expression (Fig. 6) and the site of defence responses in resistant plants, notably the xylem parenchyma cells and the cambial zone.

DISCUSSION

[0088] Many plant-pathogen interactions fit the gene-for-gene hypothesis (Flor, 1971) which states that plants are resistant when they contain an *R* gene that matches an avirulence (*Avr*) gene in the invading pathogen. Isolation of plant *R* genes and pathogen *Avr* genes has revealed little about the role their gene products play in the defence response. Structural analysis of *R* genes, that share many common features, suggests that they are active in signalling cascade(s) that coordinate initial plant defence responses to impair pathogen growth (Hammond-Kosack and Jones, 1997; Ellis and Jones, 1998). While avirulence genes probably have a role in fitness or pathogenicity of the pathogen (Leach and White, 1996; Vivian and Gibbon, 1997), *R* gene products may have a function in plant development, and hence are expressed in healthy, unchallenged plants ready to detect the attack (Hammond-Kosack and Jones, 1997). [0089] Detection of *I-2* transcripts using Northern blot analysis appeared to be difficult. However, RT-PCR analysis revealed that *I-2* and several of its homologs are expressed in roots, stems and leaves (Fig. 1B). Because amplifications were not performed quantitatively, band intensity are not likely to reflect actual expression levels, but are rather the result of differences in efficiency at which fragments are amplified. The fact that the level of *I-2* transcripts is very low, that more than one homologs are transcribed, and that based on the known sequences of *I-2* and its homologs no *I-*

2 specific probes could be designed for *in situ* hybridization, prompted us to use a promoter-*uidA* fusion for *I-2* expression analysis.

[0090] The functionality of the *I-2* promoter fragment used was tested by complementation analysis. A construct containing a 3.8 kb 5' flanking sequence of the *I-2* gene upstream of the *I-2* coding and 1.1 kb 3' downstream region conferred resistance to a susceptible line. This indicated that the 3.8 kb promoter of *I-2* is sufficient for functional expression of the *I-2* resistance gene. Only in 29% of the transgenic lines tested complementation was found. Similar functional transformation frequencies were obtained using cosmids containing the *I-2* gene (Simons et al., 1998). In transgenic lines containing the cosmid with the largest promoter sequence (B22), where external influences will be very small, resistance was found in 73% of the transgenic lines. When cosmids were used with shorter promoter sequences, frequencies at which resistant lines were found dropped. For example, with cosmid A55 that contains a promoter sequence of approximately 3 kb, only in 23% of the transgenic lines resistance was found. This suggests that for functional *I-2* expression flanking sequences of the gene are important.

[0091] Transgenic plants were generated with the 3.8 kb 5' flanking sequence of the *I-2* gene fused to the GUS reporter gene. Levels of GUS activity in these transgenic plants showed to be highly variable, supporting the observation that the *I-2* promoter activity is sensitive to position effects. The GUS activity found were in agreement with the RT-PCR, namely expression in roots, stems and leaves. RNA gel blot analyses using *RPS2*, *RPM1*, *RPP5*, *Mi*, *Pto*, *Prf*, *Xa21* and *Cf-9* as probes have revealed the presence of low abundant transcripts in unchallenged plants (Grant et al., 1995; Salemon et al., 1996; Hammond-Kosack and Jones, 1997; Parker et al., 1997; Milligan et al., 1998). This indicates that most *R* genes, and their homologs, are expressed in the absence of the corresponding *avr* expressing pathogen. *I-2* shares this common feature of *R* genes since expression of *I-2* was detected in tomato plants free of *Fusarium*. For most of the *R* genes induction of expression by the pathogen has not been observed or is not known because of the localized response. Only for the nematode resistance genes *Hs1^{Pro-1}* and the bacterial resistance gene *Xa1* induction by the pathogen has been shown (Cai et al., 1997; Yoshimura et al., 1998). In *F. oxysporum* f.sp. *lycopersici* infected tomato plants no increased *I-2* promoter driven GUS activity was found (Fig. 4). The same *I-2* promoter-*uidA* constructs were used to transform cell suspension of tomato cell line MSK8. Transgenic cell lines showed constitutively GUS activity; treatment of the cells with conidia of *F. oxysporum* f.sp. *lycopersici* race 1 or race 2, culture filtrates of *F. oxysporum* f.sp. *lycopersici* or xylanase (an aspecific elicitor) did not change the GUS activity. Taken together, these results suggest that the *I-2* expression is not regulated by pathogen infection.

[0092] Histochemical observations have revealed that GUS activity was primarily found in vascular tissue. Therefore, the relative GUS activity in roots, stems and leaves (Fig. 3) very well could reflect the relative presence of vascular cells in these tissues. The observation that *F. oxysporum* f.sp. *lycopersici* colonizes the vascular tissue of the plant, suggests that *I-2* is expressed in cells that are responsible for the resistance responses. The fungus enters the root via wounds, including natural wounds caused by lateral root formation. At the site of lateral root formation, pronounced *I-2* expression was found in a ring of cells lining at the base of the lateral root, probably outside the vascular tissue of the main root. The fungus that enters the disrupted cortex has to pass this site of *I-2* expression before it invades the vascular tissue. This might create a recognition point for the *I-2* based defences. Growing in the xylem vessels of the root *F. oxysporum* f.sp. *lycopersici* will try to spread lateral and vertical. Vertical invasion is restricted by the production of gels, gums and tyloses. These are produced by xylem parenchyma cells, which show *I-2* expression indicating that the *I-2* gene could directly mediate these responses in xylem parenchyma cells. In the lateral direction it has been proposed that cell wall depositions and the production of antifungal compounds are responsible for the resistance response, and that these resistance reactions are orchestrated by secondary xylem parenchyma cells (Beckman et al., 1989). In susceptible plants *F. oxysporum* f.sp. *lycopersici* invades the xylem parenchyma and even continues into the cambium zone (Fig. 7C and E). Plants of which the cambium zone is affected will no longer be able to bypass the infection by the creation of new xylem vessels. In resistant tomato plants lateral growth of *F. oxysporum* f.sp. *lycopersici* is prevented. This is associated with many defence responses in cells flanking the xylem vessels. Among these responses is a brownish discolourization of phenolic origin (Fig. 7D and F) (Beckman, 1987). GUS activity in xylem parenchyma cells and in cambium cells, as found, overlaps with the location where the crucial resistance mechanism is expected. Expression of *I-2* in the lateral root and adventitious root formation suggests that *I-2* might also be involved in the formation of new roots to compensate the affected absorptive and transport capacity of the root system impaired by *F. oxysporum* f.sp. *lycopersici* infection.

[0093] It is speculated that *R* gene products will activate multiple signalling pathways simultaneously such as was found for numerous mammalian receptor proteins (O'Neill, 1995; Li et al., 1997; Ellis and Jones, 1998). Furthermore, sequence homology has been found between NBS containing *R* genes and genes involved in regulation of apoptotic cell death in animals (Chinnaiyan et al., 1997; Van der Biezen and Jones, 1998). This has led to the hypothesis that *R* genes are the key components in a branched signalling pathway resulting in the induction of a wide range of responses that are directed against a broad range of pathogens. One of these branches might include the mediation of rapid host cell death. The resistance of tomato to *F. oxysporum* f.sp. *lycopersici* is accompanied by many general defence responses like callose deposition, lignification, production of phytoalexins and induction of PR proteins (Beckman, 1987;

Beckman and Roberts, 1995). Since the resistance response is mediated by xylem parenchyma cells, defence mechanisms explored by these cells will probably be close to their normal activity which involves the formation or support of xylem. Tracheary element formation is characterized by secondary wall thickening, which are subsequently lignified, and a novel form of programmed cell death resulting in mature tracheary elements (McCann, 1997; Jones and Groover, 1997). In certain situations like lateral root formation, or vascular obstruction by pathogens, cells that are already differentiated will trans-differentiate to form vascular tissue, thereby establishing continuity of water-transporting tissue (Sachs, 1981; Aloni, 1987). *I-2* expression is found in vascular cambium, xylem parenchyma cells and premature xylem vessels, tissue that can develop into tracheary elements. Furthermore, *I-2* expression was found in lateral and adventitious root formation. This all together let us to hypothesize that the *I-2* gene, or the original function of *I-2* like genes, could be involved in the acceleration of de-differentiation of tissue or in the signal transduction pathway leading to the development of vascular tissue.

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Annex to the description

[0124]

5

SEQUENCE LISTING

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| 35 | aac act cgg cgc ttg aag atg cca cta cat ctg agc agg ttg aaa agc Asn Thr Arg Arg Leu Lys Met Pro Leu His Leu Ser Arg Leu Lys Ser | 2016 |
| | 660 665 670 | |
| 40 | ctc caa gtg ttg gtg gga ccc aag ttt ttt gta gat ggt tgg aga atg Leu Gln Val Leu Val Gly Pro Lys Phe Phe Val Asp Gly Trp Arg Met | 2064 |
| | 675 680 685 | |
| 45 | gaa gat ttg ggt gaa gca caa aac tta cat gga tct cta tca gtt gtg Glu Asp Leu Gly Glu Ala Gln Asn Leu His Gly Ser Leu Ser Val Val | 2112 |
| | 690 695 700 | |
| 50 | aag ttg gaa aat gtg gtt gat aga agg gaa gct gtg aag gca aag atg Lys Leu Glu Asn Val Val Asp Arg Arg Glu Ala Val Lys Ala Lys Met | 2160 |
| | 705 710 715 720 | |
| 55 | agg gag aag aat cat gtt gag caa tta tca ttg gag tgg agt gaa agt Arg Glu Lys Asn His Val Glu Gln Leu Ser Leu Glu Trp Ser Glu Ser | 2208 |
| | 725 730 735 | |
| 60 | agt att gcc gac aat tca caa aca gaa agt gac ata ctt gat gag cta Ser Ile Ala Asp Asn Ser Gln Thr Glu Ser Asp Ile Leu Asp Glu Leu | 2256 |
| | 740 745 750 | |
| 65 | tgc cca cat aaa aac atc aaa aaa gtc gaa atc agt gga tat aga ggg Cys Pro His Lys Asn Ile Lys Lys Val Glu Ile Ser Gly Tyr Arg Gly | 2304 |
| | 755 760 765 | |

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| | | |
|----|---|------|
| 5 | aca aac ttt ccc aat tgg gta gct gat cct ttg ttt ctt aag ctg gtg Thr Asn Phe Pro Asn Trp Val Ala Asp Pro Leu Phe Leu Lys Leu Val 770 775 780 | 2352 |
| 10 | aat ttg tct cta aga aac tgc aag gac tgt tac tcc ttg cca gca cta Asn Leu Ser Leu Arg Asn Cys Lys Asp Cys Tyr Ser Leu Pro Ala Leu 785 790 795 800 | 2400 |
| 15 | gga caa ctc cct tgt ttg aaa ttc ctt tcc gtt aaa ggg atg cat gga Gly Gln Leu Pro Cys Leu Lys Phe Leu Ser Val Lys Gly Met His Gly 805 810 815 | 2448 |
| 20 | ata aga gtg gtg acg gaa gaa ttc tat ggc aga ttg tcc tcc aaa aag Ile Arg Val Val Thr Glu Glu Phe Tyr Gly Arg Leu Ser Ser Lys Lys 820 825 830 | 2496 |
| 25 | cct ttt aac tct cta gag aag ctt gaa ttt gaa gat atg acg gag tgg Pro Phe Asn Ser Leu Glu Lys Leu Glu Phe Glu Asp Met Thr Glu Trp 835 840 845 | 2544 |
| 30 | aag caa tgg cac gca cta gga att gga gag ttc cct aca ctt gag aac Lys Gln Trp His Ala Leu Gly Ile Gly Glu Phe Pro Thr Leu Glu Asn 850 855 860 | 2592 |
| 35 | ctt tca att aaa aat tgc cct gag ctc agt ttg gag ata ccc atc caa Leu Ser Ile Lys Asn Cys Pro Glu Leu Ser Leu Glu Ile Pro Ile Gln 865 870 875 880 | 2640 |
| 40 | ttt tca agt tta aaa agg tta gaa gtt agt gat tgt cca gtt gtt ttt Phe Ser Ser Leu Lys Arg Leu Glu Val Ser Asp Cys Pro Val Val Phe 885 890 895 | 2688 |
| 45 | gat gat gcc caa ctg ttt aga tcc caa ctt gag gca atg aag cag att Asp Asp Ala Gln Leu Phe Arg Ser Gln Leu Glu Ala Met Lys Gln Ile 900 905 910 | 2736 |
| 50 | gag gaa ata gat ata tgt gat tgt aac tct gtt acc tcc ttt cct ttt Glu Glu Ile Asp Ile Cys Asp Cys Asn Ser Val Thr Ser Phe Pro Phe 915 920 925 | 2784 |
| 55 | agc ata ctg cca act acc ttg aag aga ata cag ata tct cgt tgc cca Ser Ile Leu Pro Thr Thr Leu Lys Arg Ile Gln Ile Ser Arg Cys Pro 930 935 940 | 2832 |
| 60 | aaa ttg aaa ttg gag gcg cca gtt ggt gag atg ttt gtg gag tat ttg Lys Leu Lys Leu Glu Ala Pro Val Gly Glu Met Phe Val Glu Tyr Leu 945 950 955 960 | 2880 |
| 65 | aga gtg aat gat tgt ggt tgt gta gat gat ata tca cct gag ttt ctc Arg Val Asn Asp Cys Gly Cys Val Asp Asp Ile Ser Pro Glu Phe Leu 965 970 975 | 2928 |

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| | | |
|----|---|------|
| | cca aca gca cgt caa ttg agt att gaa aat tgc cag aac gtt act agg | 2976 |
| | Pro Thr Ala Arg Gln Leu Ser Ile Glu Asn Cys Gln Asn Val Thr Arg | |
| 5 | 980 985 990 | |
| | ttt ttg att cct act gcc act gaa act ctc cgt att tcg aat tgt gag | 3024 |
| | Phe Leu Ile Pro Thr Ala Thr Glu Thr Leu Arg Ile Ser Asn Cys Glu | |
| | 995 1000 1005 | |
| 10 | aat gtt gaa aaa cta tcg gtg gca tgt gga gga gcg gcc cag atg acg | 3072 |
| | Asn Val Glu Lys Leu Ser Val Ala Cys Gly Gly Ala Ala Gln Met Thr | |
| | 1010 1015 1020 | |
| 15 | tca ctg aat att tgg gga tgt aag aag ctc aag tgt ctt cca gaa ctc | 3120 |
| | Ser Leu Asn Ile Trp Gly Cys Lys Lys Leu Lys Cys Leu Pro Glu Leu | |
| | 1025 1030 1035 1040 | |
| 20 | ctt cca tct ctc aag gaa ctg cgt ctg tct gat tgt cca gaa ata gaa | 3168 |
| | Leu Pro Ser Leu Lys Glu Leu Arg Leu Ser Asp Cys Pro Glu Ile Glu | |
| | 1045 1050 1055 | |
| 25 | gga gaa ttg ccc ttc aat tta gaa ata ctc cgt atc ata tat tgc aag | 3216 |
| | Gly Glu Leu Pro Phe Asn Leu Glu Ile Leu Arg Ile Ile Tyr Cys Lys | |
| | 1060 1065 1070 | |
| | aaa ctg gtg aat ggc cga aag gag tgg cat tta cag aga ctc aca gag | 3264 |
| | Lys Leu Val Asn Gly Arg Lys Glu Trp His Leu Gln Arg Leu Thr Glu | |
| | 1075 1080 1085 | |
| 30 | tta tgg atc gat cat gat ggg agt gac gaa gat att gaa cat tgg gag | 3312 |
| | Leu Trp Ile Asp His Asp Gly Ser Asp Glu Asp Ile Glu His Trp Glu | |
| | 1090 1095 1100 | |
| 35 | ttg cct tgt tct att cag aga ctt acc ata aag aat ctt aaa aca tta | 3360 |
| | Leu Pro Cys Ser Ile Gln Arg Leu Thr Ile Lys Asn Leu Lys Thr Leu | |
| | 1105 1110 1115 1120 | |
| 40 | agc agc caa cat ctc aaa agc ctc acc tct ctt caa tat cta tgt att | 3408 |
| | Ser Ser Gln His Leu Lys Ser Leu Thr Ser Leu Gln Tyr Leu Cys Ile | |
| | 1125 1130 1135 | |
| 45 | gag ggt tat tta tct cag att cag tca caa ggc cag ctt tcc tcc ttt | 3456 |
| | Glu Gly Tyr Leu Ser Gln Ile Gln Ser Gln Gly Gln Leu Ser Ser Phe | |
| | 1140 1145 1150 | |
| 50 | tct cac ctc act tcg ctt caa act cta caa atc tgg aat ttc ctt aat | 3504 |
| | Ser His Leu Thr Ser Leu Gln Thr Leu Gln Ile Trp Asn Phe Leu Asn | |
| | 1155 1160 1165 | |
| 55 | ctc caa tca ctt gct gaa tca gca ctg ccc tcc tcc ctc tct cac ctg | 3552 |
| | Leu Gln Ser Leu Ala Glu Ser Ala Leu Pro Ser Ser Leu Ser His Leu | |
| | 1170 1175 1180 | |

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gag ata gat gat tgc cct aat ctc caa tca ctc ttc gaa tca gca ctg 3600
 Glu Ile Asp Asp Cys Pro Asn Leu Gln Ser Leu Phe Glu Ser Ala Leu
 5 1185 1190 1195 1200

ccc tcc tcc ctc tct cag ctg ttc atc cag gat tgc cct aat ctc caa 3648
 Pro Ser Ser Leu Ser Gln Leu Phe Ile Gln Asp Cys Pro Asn Leu Gln
 1205 1210 1215

10 tcc ctt cca ttt aaa ggg atg ccc tct tcc ctc tct aaa cta tct att 3696
 Ser Leu Pro Phe Lys Gly Met Pro Ser Ser Leu Ser Lys Leu Ser Ile
 1220 1225 1230

15 ttc aat tgc cca ttg ctc aca cca cta cta gaa ttt gac aag ggg gaa 3744
 Phe Asn Cys Pro Leu Leu Thr Pro Leu Leu Glu Phe Asp Lys Gly Glu
 1235 1240 1245

20 tac tgg cca caa att gct cat att ccc atc ata aat atc gat tgg aaa 3792
 Tyr Trp Pro Gln Ile Ala His Ile Pro Ile Ile Asn Ile Asp Trp Lys
 1250 1255 1260

tat att taa 3801
 Tyr Ile
 25 1265

<210> 5
 <211> 1267
 <212> PRT
 30 <213> Fusarium oxysporum

<400> 5
 Met Glu Ile Gly Leu Ala Val Gly Gly Ala Phe Leu Ser Ser Ala Leu
 1 5 10 15
 35 Asn Val Leu Phe Asp Arg Leu Ala Pro Asn Gly Asp Leu Leu Asn Met
 20 25 30

40 Phe Arg Lys His Lys Asp His Val Lys Leu Leu Lys Lys Leu Lys Met
 35 40 45

Thr Leu Arg Gly Ile Gln Ile Val Leu Ser Asp Ala Glu Asn Lys Gln
 50 55 60

45 Ala Ser Asn Pro Ser Val Arg Asp Trp Leu Asn Glu Leu Arg Asp Ala
 65 70 75 80

Val Asp Ser Ala Glu Asn Leu Ile Glu Glu Val Asn Tyr Glu Ala Leu
 85 90 95

50 Arg Leu Lys Val Glu Gly Gln His Gln Asn Phe Ser Glu Thr Ser Asn
 100 105 110

55

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Gln Gln Val Ser Asp Asp Phe Phe Leu Asn Ile Lys Asp Lys Leu Glu
 115 120 125
 5 Asp Thr Ile Glu Thr Leu Lys Asp Leu Gln Glu Gln Ile Gly Leu Leu
 130 135 140
 Gly Leu Lys Glu Tyr Phe Asp Ser Thr Lys Leu Glu Thr Arg Arg Pro
 10 145 150 155 160
 Ser Thr Ser Val Asp Asp Glu Ser Asp Ile Phe Gly Arg Gln Ser Glu
 165 170 175
 15 Ile Glu Asp Leu Ile Asp Arg Leu Leu Ser Glu Gly Ala Ser Gly Lys
 180 185 190
 Lys Leu Thr Val Val Pro Ile Val Gly Met Gly Gly Gln Gly Lys Thr
 195 200 205
 20 Thr Leu Ala Lys Ala Val Tyr Asn Asp Glu Arg Val Lys Asn His Phe
 210 215 220
 25 Asp Leu Lys Ala Trp Tyr Cys Val Ser Glu Gly Phe Asp Ala Leu Arg
 225 230 235 240
 Ile Thr Lys Glu Leu Leu Gln Glu Ile Gly Lys Phe Asp Ser Lys Asp
 245 250 255
 30 Val His Asn Asn Leu Asn Gln Leu Gln Val Lys Leu Lys Glu Ser Leu
 260 265 270
 Lys Gly Lys Lys Phe Leu Ile Val Leu Asp Asp Val Trp Asn Glu Asn
 275 280 285
 35 Tyr Asn Glu Trp Asn Asp Leu Arg Asn Ile Phe Ala Gln Gly Asp Ile
 290 295 300
 40 Gly Ser Lys Ile Ile Val Thr Thr Arg Lys Asp Ser Val Ala Leu Met
 305 310 315 320
 Met Gly Asn Glu Gln Ile Arg Met Gly Asn Leu Ser Thr Glu Ala Ser
 325 330 335
 45 Trp Ser Leu Phe Gln Arg His Ala Phe Glu Asn Met Asp Pro Met Gly
 340 345 350
 His Pro Glu Leu Glu Glu Val Gly Arg Gln Ile Ala Ala Lys Cys Lys
 355 360 365
 50 Gly Leu Pro Leu Ala Leu Lys Thr Leu Ala Gly Met Leu Arg Ser Lys
 370 375 380

55

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5 Ser Glu Val Glu Glu Trp Lys Arg Ile Leu Arg Ser Glu Ile Trp Glu
 385 390 395 400
 Leu Pro His Asn Asp Ile Leu Pro Ala Leu Met Leu Ser Tyr Asn Asp
 405 410 415
 10 Leu Pro Ala His Leu Lys Arg Cys Phe Ser Phe Cys Ala Ile Phe Pro
 420 425 430
 Lys Asp Tyr Pro Phe Arg Lys Glu Gln Val Ile His Leu Trp Ile Ala
 435 440 445
 15 Asn Gly Leu Val Pro Val Lys Asp Glu Ile Asn Gln Asp Leu Gly Asn
 450 455 460
 20 Gln Tyr Phe Leu Glu Leu Arg Ser Arg Ser Leu Phe Glu Lys Val Pro
 465 470 475 480
 Asn Pro Ser Lys Arg Asn Ile Glu Glu Leu Phe Leu Met His Asp Leu
 485 490 495
 25 Val Asn Asp Leu Ala Gln Leu Ala Ser Ser Lys Leu Cys Ile Arg Leu
 500 505 510
 Glu Glu Ser Gln Gly Ser His Met Leu Glu Gln Cys Arg His Leu Ser
 515 520 525
 30 Tyr Ser Ile Gly Phe Asn Gly Glu Phe Lys Lys Leu Thr Pro Leu Tyr
 530 535 540
 Lys Leu Glu Gln Leu Arg Thr Leu Leu Pro Ile Arg Ile Glu Phe Arg
 545 550 555 560
 Leu His Asn Leu Ser Lys Arg Val Leu His Asn Ile Leu Pro Thr Leu
 565 570 575
 40 Arg Ser Leu Arg Ala Leu Ser Phe Ser Gln Tyr Lys Ile Lys Glu Leu
 580 585 590
 Pro Asn Asp Leu Phe Thr Lys Leu Lys Leu Leu Arg Phe Leu Asp Ile
 595 600 605
 45 Ser Arg Thr Trp Ile Thr Lys Leu Pro Asp Ser Ile Cys Gly Leu Tyr
 610 615 620
 Asn Leu Glu Thr Leu Leu Leu Ser Ser Cys Ala Asp Leu Glu Glu Leu
 625 630 635 640
 Pro Leu Gln Met Glu Lys Leu Ile Asn Leu Arg His Leu Asp Val Ser
 645 650 655
 55

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Asn Thr Arg Arg Leu Lys Met Pro Leu His Leu Ser Arg Leu Lys Ser
 660 665 670
 5
 Leu Gln Val Leu Val Gly Pro Lys Phe Phe Val Asp Gly Trp Arg Met
 675 680 685
 Glu Asp Leu Gly Glu Ala Gln Asn Leu His Gly Ser Leu Ser Val Val
 690 695 700
 10
 Lys Leu Glu Asn Val Val Asp Arg Arg Glu Ala Val Lys Ala Lys Met
 705 710 715 720
 15
 Arg Glu Lys Asn His Val Glu Gln Leu Ser Leu Glu Trp Ser Glu Ser
 725 730 735
 Ser Ile Ala Asp Asn Ser Gln Thr Glu Ser Asp Ile Leu Asp Glu Leu
 740 745 750
 20
 Cys Pro His Lys Asn Ile Lys Lys Val Glu Ile Ser Gly Tyr Arg Gly
 755 760 765
 Thr Asn Phe Pro Asn Trp Val Ala Asp Pro Leu Phe Leu Lys Leu Val
 770 775 780
 25
 Asn Leu Ser Leu Arg Asn Cys Lys Asp Cys Tyr Ser Leu Pro Ala Leu
 785 790 795 800
 30
 Gly Gln Leu Pro Cys Leu Lys Phe Leu Ser Val Lys Gly Met His Gly
 805 810 815
 Ile Arg Val Val Thr Glu Glu Phe Tyr Gly Arg Leu Ser Ser Lys Lys
 820 825 830
 35
 Pro Phe Asn Ser Leu Glu Lys Leu Glu Phe Glu Asp Met Thr Glu Trp
 835 840 845
 40
 Lys Gln Trp His Ala Leu Gly Ile Gly Glu Phe Pro Thr Leu Glu Asn
 850 855 860
 Leu Ser Ile Lys Asn Cys Pro Glu Leu Ser Leu Glu Ile Pro Ile Gln
 865 870 875 880
 45
 Phe Ser Ser Leu Lys Arg Leu Glu Val Ser Asp Cys Pro Val Val Phe
 885 890 895
 Asp Asp Ala Gln Leu Phe Arg Ser Gln Leu Glu Ala Met Lys Gln Ile
 900 905 910
 50
 Glu Glu Ile Asp Ile Cys Asp Cys Asn Ser Val Thr Ser Phe Pro Phe
 915 920 925
 55

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Ser Ile Leu Pro Thr Thr Leu Lys Arg Ile Gln Ile Ser Arg Cys Pro
 930 935 940
 5
 Lys Leu Lys Leu Glu Ala Pro Val Gly Glu Met Phe Val Glu Tyr Leu
 945 950 955 960
 Arg Val Asn Asp Cys Gly Cys Val Asp Asp Ile Ser Pro Glu Phe Leu
 10 965 970 975
 Pro Thr Ala Arg Gln Leu Ser Ile Glu Asn Cys Gln Asn Val Thr Arg
 980 985 990
 15 Phe Leu Ile Pro Thr Ala Thr Glu Thr Leu Arg Ile Ser Asn Cys Glu
 995 1000 1005
 Asn Val Glu Lys Leu Ser Val Ala Cys Gly Gly Ala Ala Gln Met Thr
 1010 1015 1020
 20 Ser Leu Asn Ile Trp Gly Cys Lys Lys Leu Lys Cys Leu Pro Glu Leu
 1025 1030 1035 1040
 Leu Pro Ser Leu Lys Glu Leu Arg Leu Ser Asp Cys Pro Glu Ile Glu
 25 1045 1050 1055
 Gly Glu Leu Pro Phe Asn Leu Glu Ile Leu Arg Ile Ile Tyr Cys Lys
 1060 1065 1070
 30 Lys Leu Val Asn Gly Arg Lys Glu Trp His Leu Gln Arg Leu Thr Glu
 1075 1080 1085
 Leu Trp Ile Asp His Asp Gly Ser Asp Glu Asp Ile Glu His Trp Glu
 1090 1095 1100
 35 Leu Pro Cys Ser Ile Gln Arg Leu Thr Ile Lys Asn Leu Lys Thr Leu
 1105 1110 1115 1120
 Ser Ser Gln His Leu Lys Ser Leu Thr Ser Leu Gln Tyr Leu Cys Ile
 40 1125 1130 1135
 Glu Gly Tyr Leu Ser Gln Ile Gln Ser Gln Gly Gln Leu Ser Ser Phe
 1140 1145 1150
 45 Ser His Leu Thr Ser Leu Gln Thr Leu Gln Ile Trp Asn Phe Leu Asn
 1155 1160 1165
 Leu Gln Ser Leu Ala Glu Ser Ala Leu Pro Ser Ser Leu Ser His Leu
 1170 1175 1180
 50 Glu Ile Asp Asp Cys Pro Asn Leu Gln Ser Leu Phe Glu Ser Ala Leu
 185 1190 1195 1200
 55

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Pro Ser Ser Leu Ser Gln Leu Phe Ile Gln Asp Cys Pro Asn Leu Gln
 1205 1210 1215
 5 Ser Leu Pro Phe Lys Gly Met Pro Ser Ser Leu Ser Lys Leu Ser Ile
 1220 1225 1230
 Phe Asn Cys Pro Leu Leu Thr Pro Leu Leu Glu Phe Asp Lys Gly Glu
 1235 1240 1245
 10 Tyr Trp Pro Gln Ile Ala His Ile Pro Ile Ile Asn Ile Asp Trp Lys
 1250 1255 1260
 15 Tyr Ile
 1265

Claims

- 20 1. Nucleotide sequence having a regulatory activity on the transcription of the 1-2 resistance gene in determined plant host cells, which nucleotide sequence comprises SEQUENCE ID NO:1.
- 25 2. Nucleotide sequence according to claim 1 which is SEQUENCE ID NO:1.
3. Regulatory nucleotide sequence according to claim 1 or 2 or a fragment thereof capable of driving a tissue specific transcription of a determined nucleic acid sequence placed under its control.
- 30 4. Recombinant nucleotide sequence comprising a nucleotide sequence according to claim 1 or 2 functionally linked to a determined nucleic acid sequence.
5. Recombinant nucleotide sequence according to claim 4, wherein the nucleic acid sequence functionally linked is a sequence encoding the I-2 resistance polypeptide.
- 35 6. Recombinant nucleotide sequence according to claim 4 or 5, which is transcribed in a tissue specific manner in plant host cells.
7. Recombinant nucleotide sequence according to anyone of claims 4 to 6, wherein the nucleic acid sequence functionally linked to the regulatory sequence is chosen among sequences expressed in a tissue specific manner in their natural hosts.
- 40 8. Recombinant nucleotide sequence according to anyone of claims 4 to 7, which is expressed in a tissue specific manner when it is transferred into a plant.
- 45 9. Recombinant nucleotide sequence according to anyone of claims 4 to 8, which is capable of being expressed in plant vascular tissue.
10. Recombinant nucleotide sequence according to anyone of claims 4 to 8, which is capable of being expressed in plant lateral root meristem.
- 50 11. Recombinant nucleotide sequence according to anyone of claims 4 to 10 which further comprises a 3' non translated sequence comprising a transcription termination codon.
- 55 12. Recombinant nucleotide sequence according to anyone of claims 4 to 11, wherein the nucleic acid sequence functionally linked is a sequence of a gene having activity in mediation of resistance to a plant pathogen when the recombinant nucleotide sequence is operatively transferred to a host plant susceptible to said plant pathogen.
13. Recombinant nucleotide sequence according to claim 12, wherein the plant pathogen is a root pathogen.

14. Recombinant nucleotide sequence according to anyone of claims 12 to 13 wherein the plant pathogen is a fungus, especially of *Fusarium* species.

15. Recombinant vector comprising a nucleotide sequence according to anyone of claims 1 to 3 or recombinant nucleotide sequence according to anyone of claims 4 to 14.

16. Recombinant vector according to claim 15, which is suitable for transforming plant cells or plants.

17. Recombinant cell comprising a nucleotide sequence according to anyone of claims 1 to 3 or recombinant nucleotide sequence according to anyone of claims 4 to 14 or a recombinant vector according to claim 15 or 16.

18. Recombinant cell according to claim 17, which is a plant cell.

19. Recombinant cell according to claim 18, which is a plant root cell or a stem cell or a leaf cell.

20. Plant which is recombined with a nucleotide sequence according to anyone of claims 1 to 3 or a recombinant nucleotide sequence according to anyone of claims 4 to 14 or a vector according to claims 15 or 16.

21. Recombinant plant according to claim 20 which is capable of expressing a gene mediating resistance to a plant pathogen chosen among fungi, in a tissue specific manner.

22. Recombinant plant according to claim 20, which is capable of preventing the infection by a plant pathogen chosen among fungi.

23. Recombinant plant according to anyone of claims 20 to 22, which is a tomato, egg plant, potato, melon, tobacco, Arabidopsis.

24. Use of a nucleotide sequence according to anyone of claims 1 to 14 or of a vector according to anyone of claims 15 or 16 or use of a part of said nucleotide sequence for the tissue specific regulation of the transcription of the determined nucleic acid sequence placed under its control.

25. Use according to claim 24 wherein the nucleotide sequence having a transcription regulatory activity is SEQUENCE ID NO: 2 or a fragment thereof sufficient to drive the transcription of a determined nucleic acid sequence placed under its control.

26. Use according to claim 24 wherein the nucleotide sequence having a transcription regulatory activity is all or part of SEQUENCE ID NO: 3.

27. Use according to anyone of claims 24 to 26, wherein the transcription regulatory activity is obtained in vascular tissue.

28. Use according to anyone of claims 24 to 26, wherein the transcription regulatory activity is obtained in the lateral root meristem tissue.

29. Process for obtaining plants having reduced susceptibility to a plant pathogen, comprising the following steps:

- i) inserting into the genome of a plant cell a nucleotide sequence according to anyone of claims 1 to 14, or a vector according to any of claims 15 or 16,
- ii) obtaining transformed plant cells,
- iii) regenerating from said transformed plant cells genetically transformed plants, and
- iv) optionally, propagating said plants.

30. Process according to claim 24 further comprising selecting transformed plants having reduced susceptibility to said plant pathogen.

31. Process for protecting plants in cultivation against plant pathogen infection, which comprises:

- i) providing the genome of plants with a nucleotide sequence according to anyone of claims 1 to 14, or a vector

according to any of claims 15 or 16, and
ii) growing said plants.

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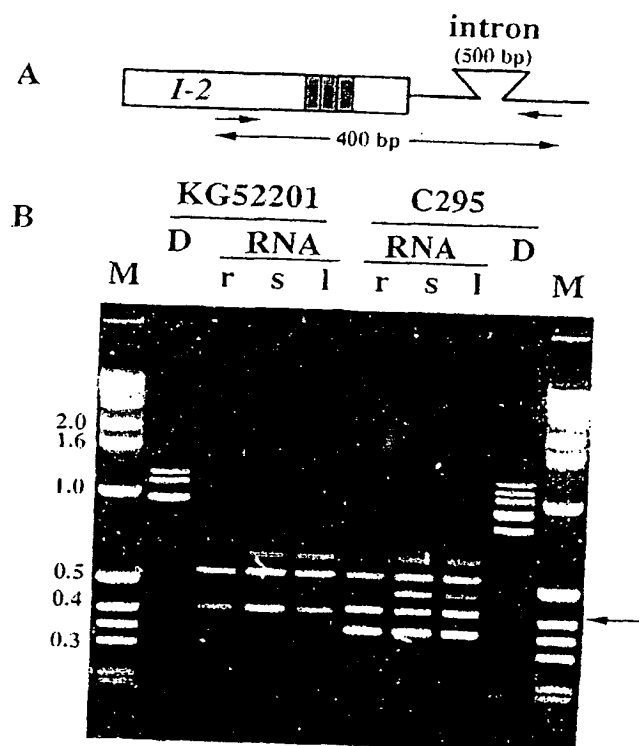
40

45

50

55

FIGURE 1



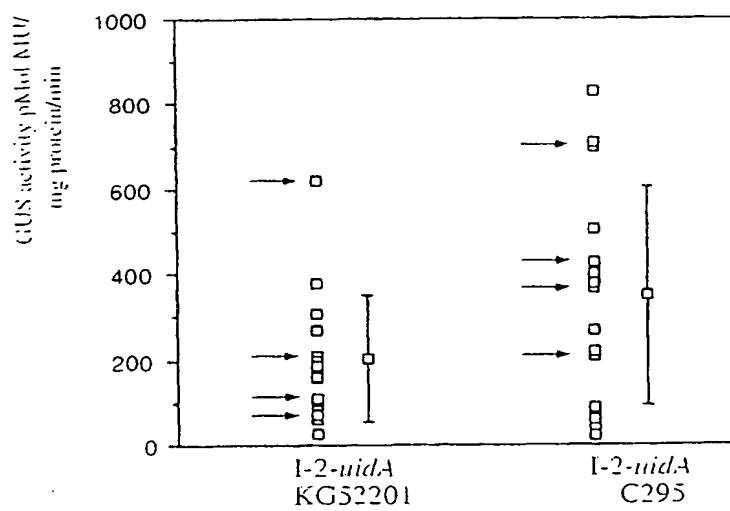


FIGURE 2

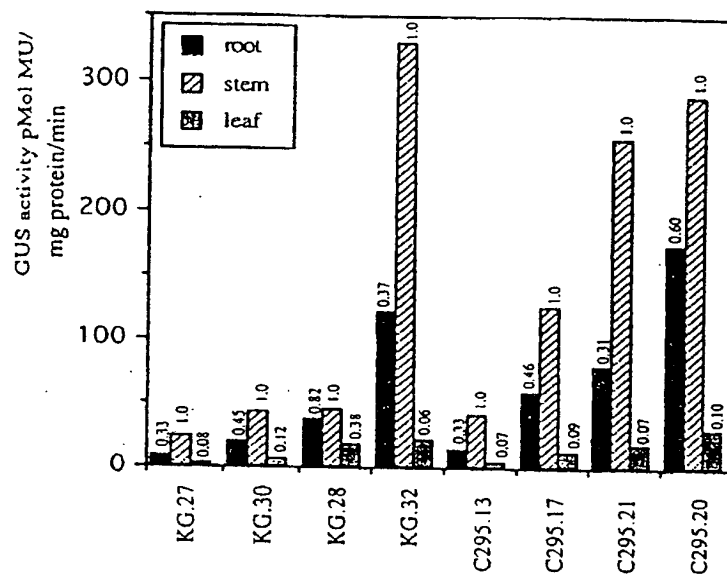


FIGURE 3A

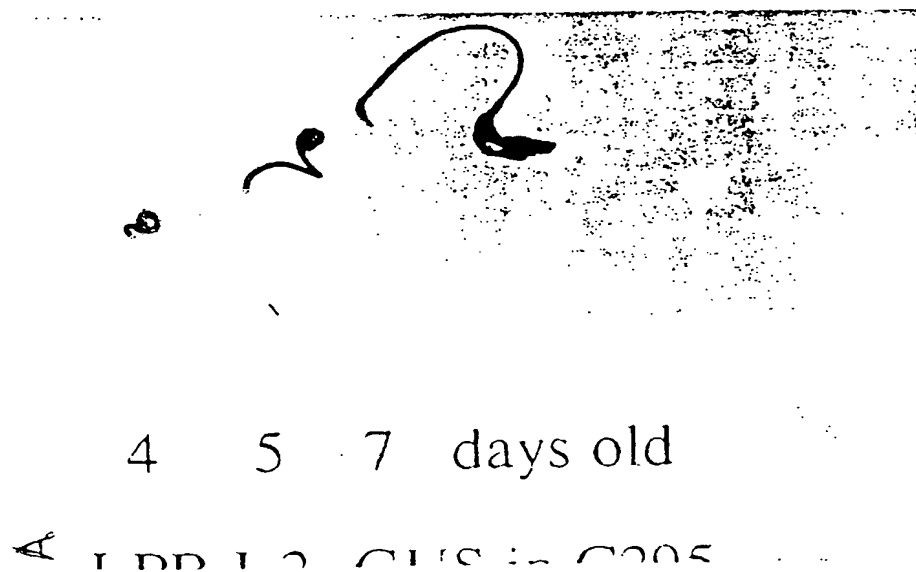


FIGURE 3B(1)

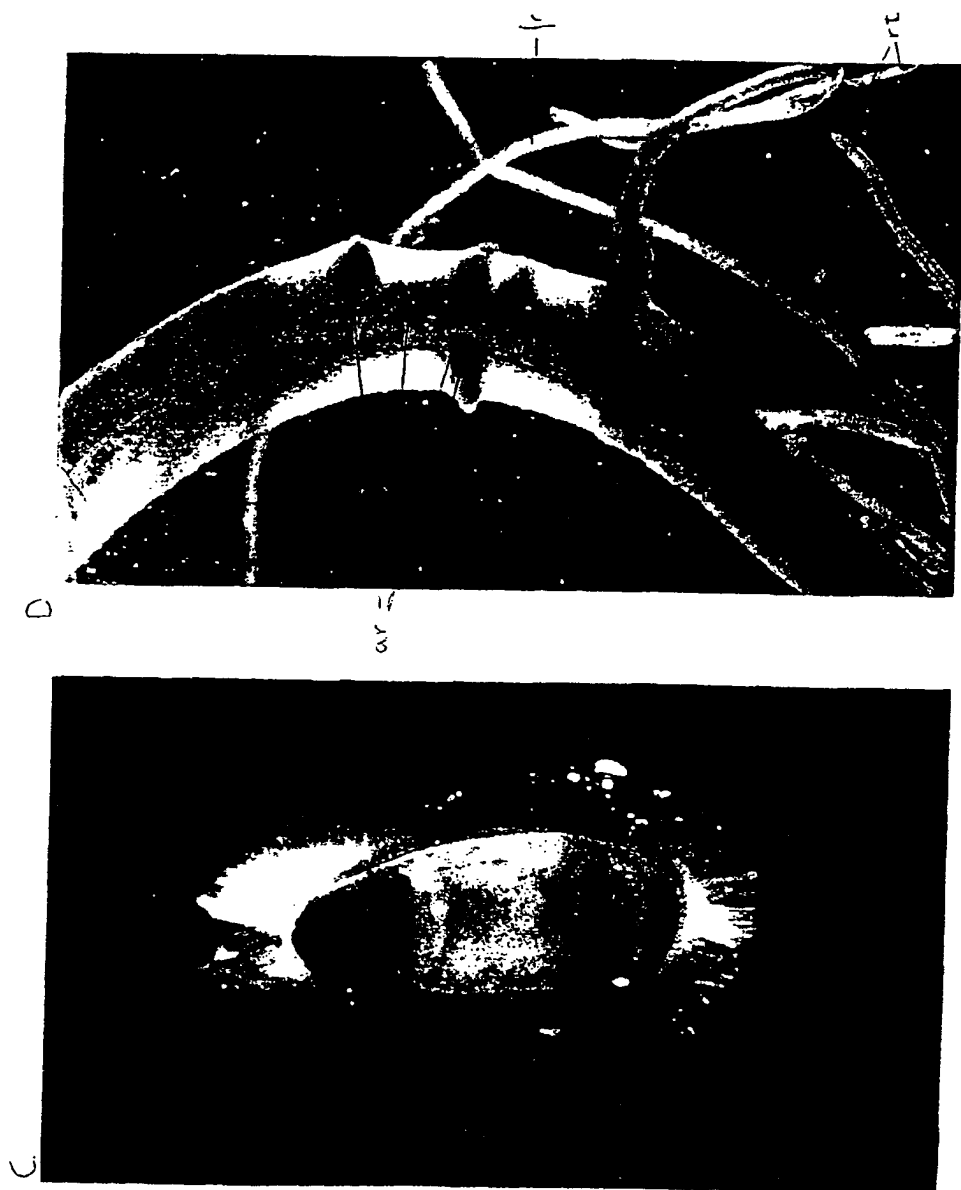


FIGURE 38(2)

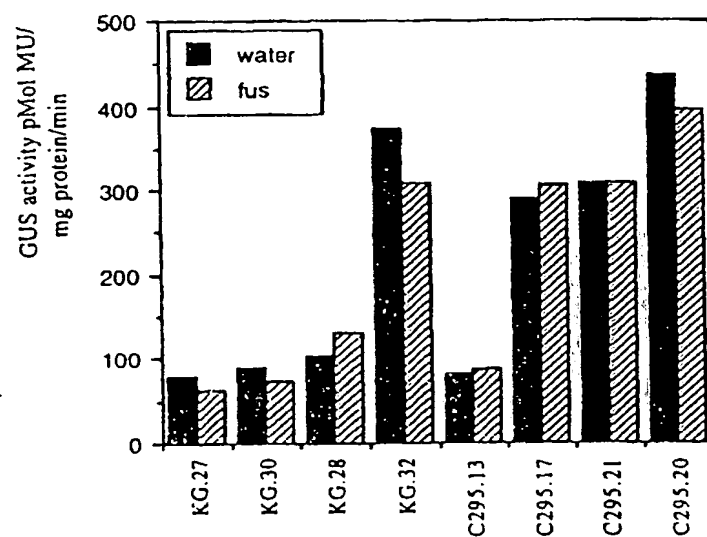


FIGURE 4

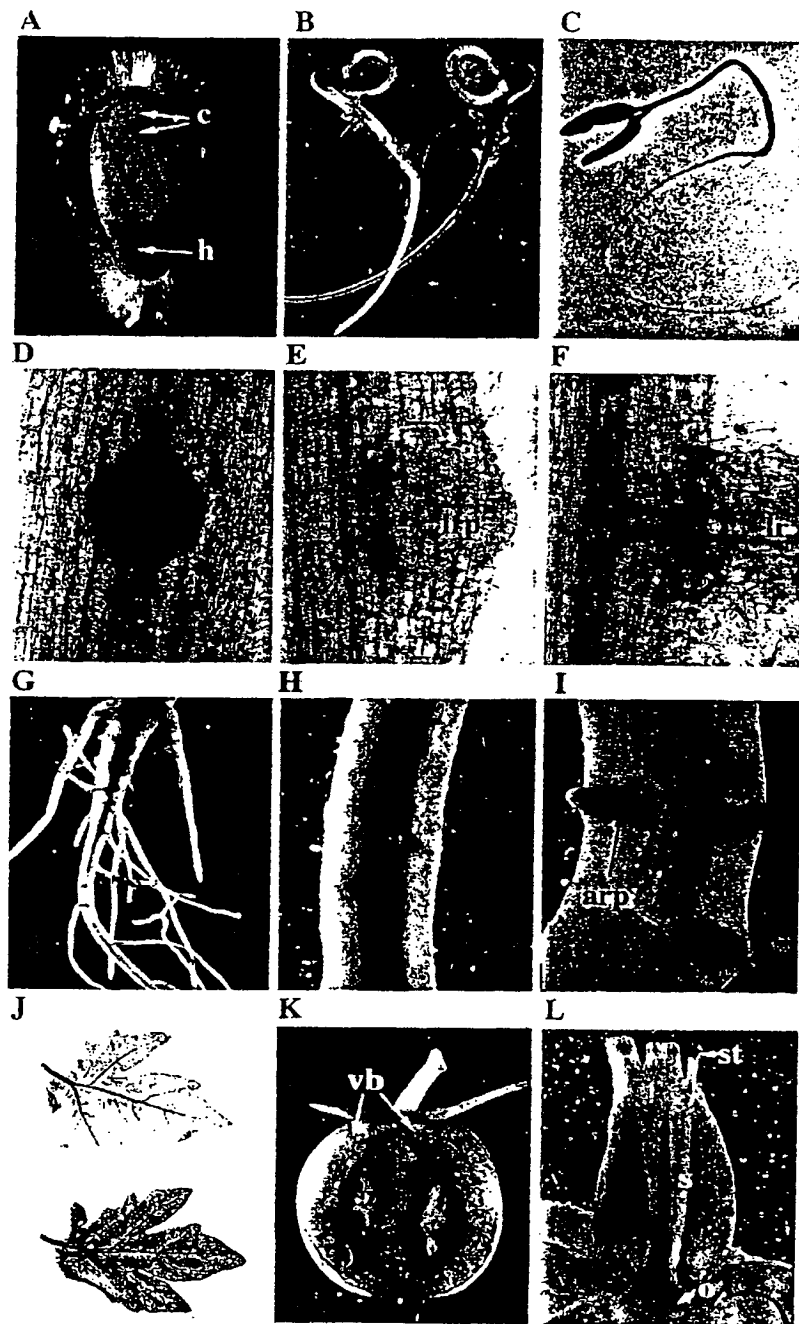


FIGURE 5

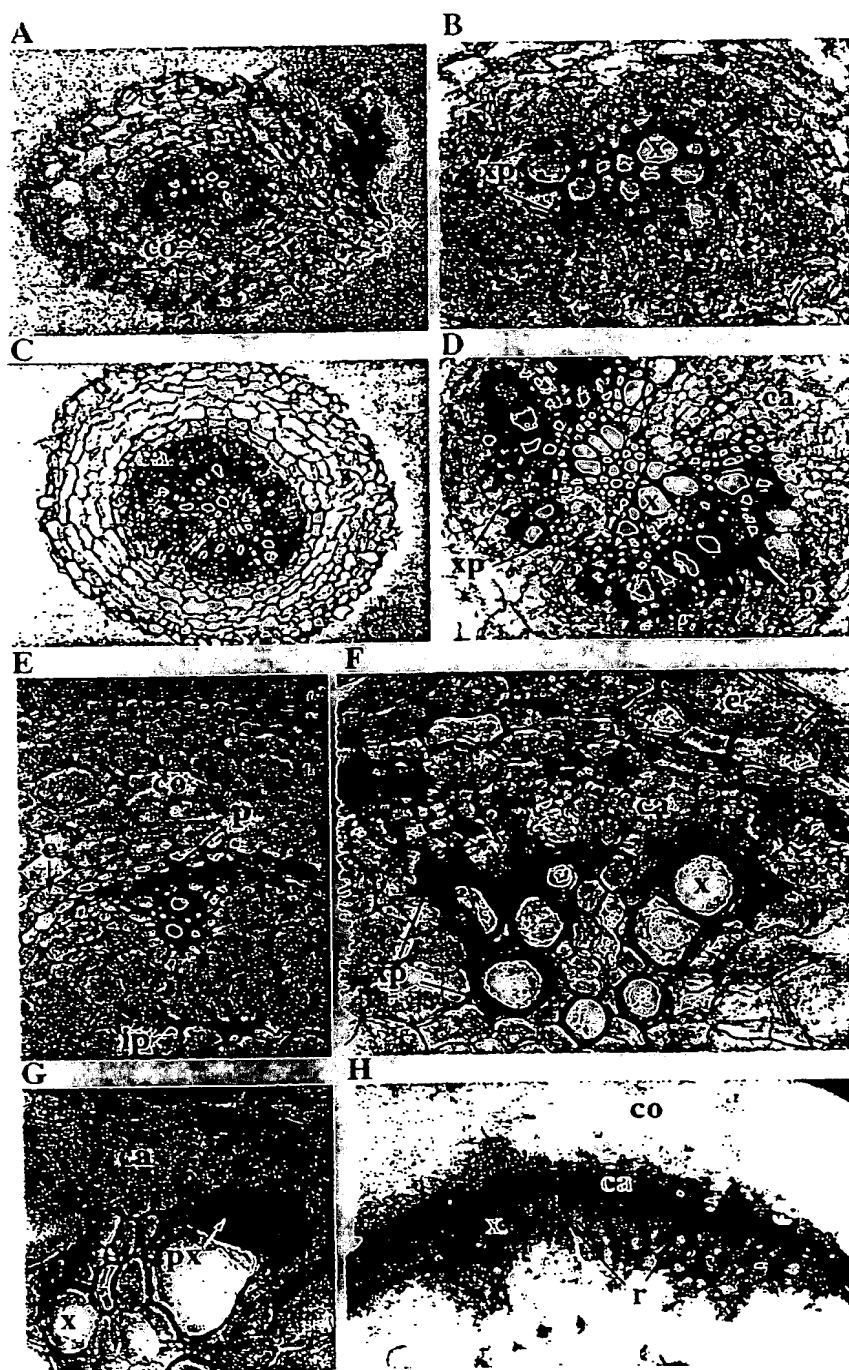


FIGURE 6

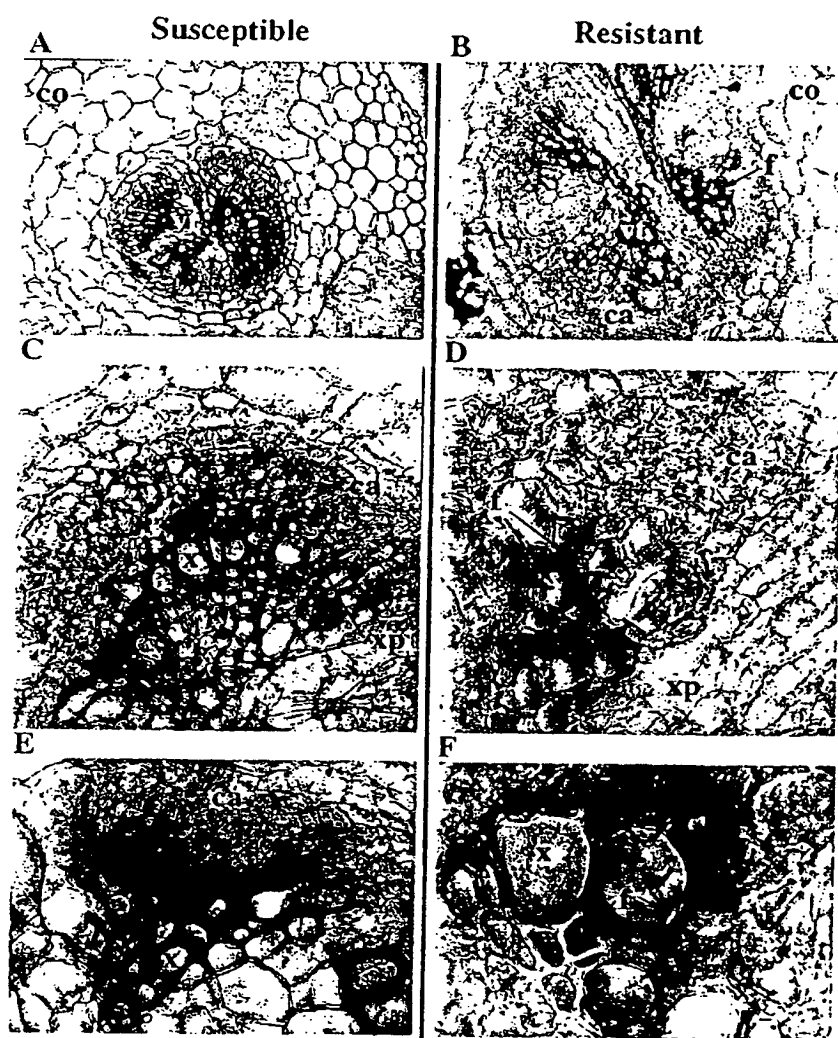


FIGURE 7

FIGURE 8A

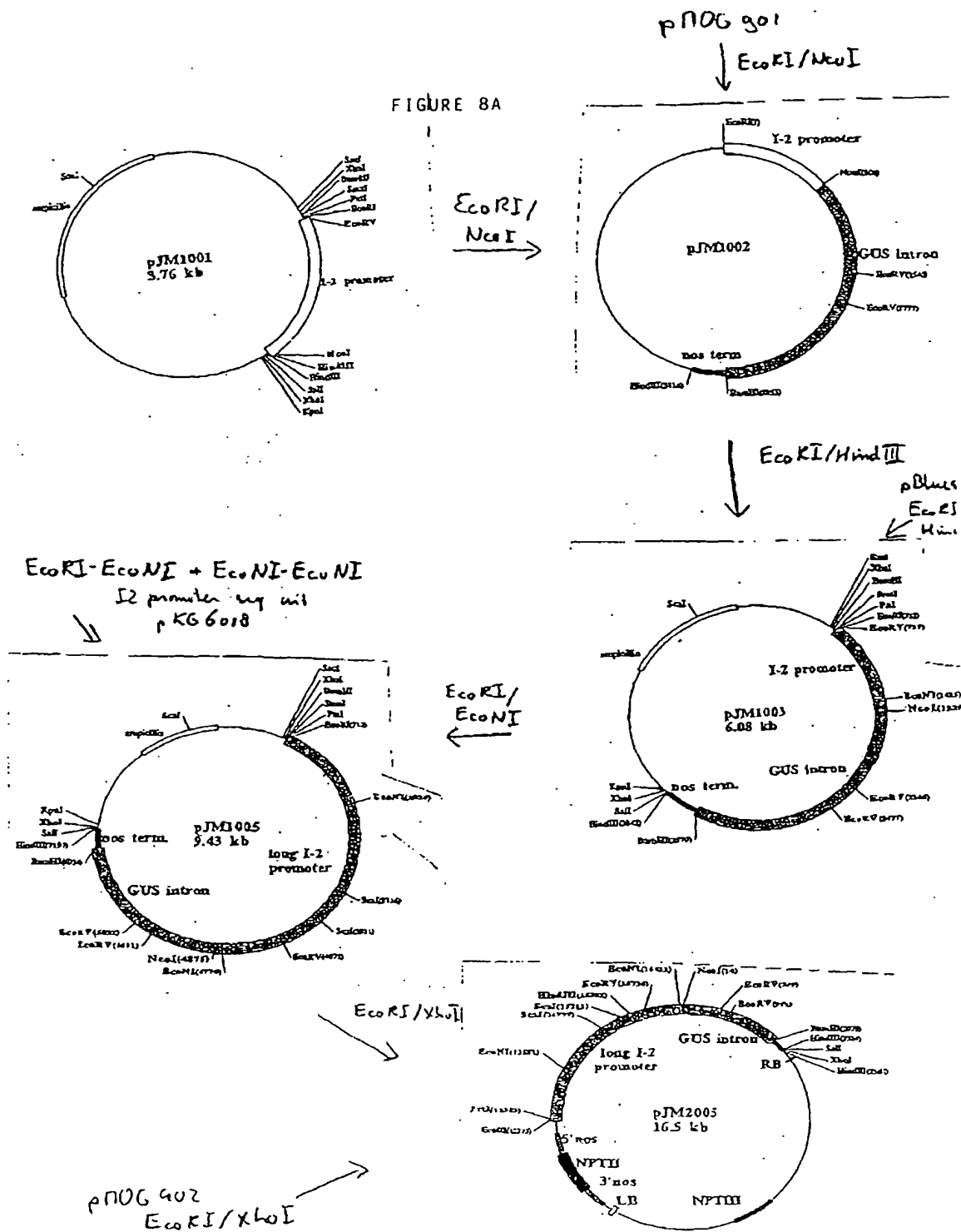
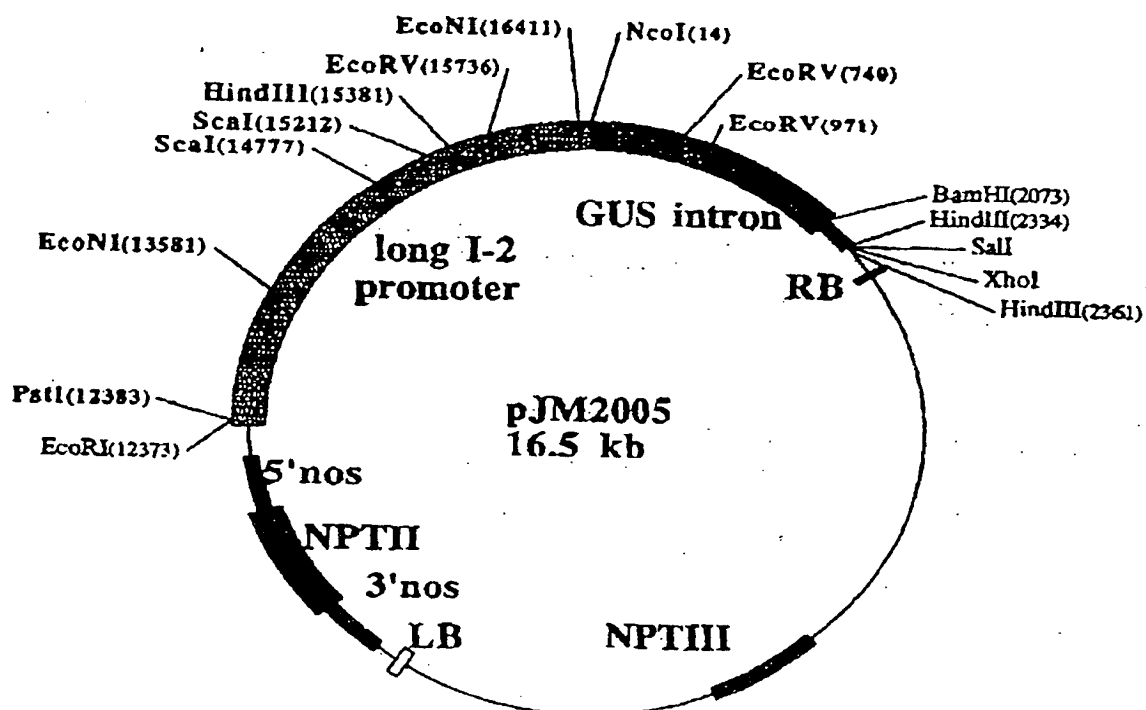


FIGURE 8B



GAATTCCTGCAGGTGGAATATACAGCCGTTGTTAGAGAAACAACCTTTGGTTATCCAAGGTGCTGGAGCAGGAAATGAA
 CCTTGCAACATTTTGACTACTAGTACATCTCAACATTCACACAAGACAATTGACTGAATCCCGTGAAAACTTGCTCTCC
 GCCTAATGAATACAGAACACCAATACTTTAGCTTTGTTTTATCATTTTCCAAAAAAGTACATCAAAAACAGCATAGCC
 TCACGCCATTTGACTTGTACTATACAACCTGACAGCACAATCGCAAAAGAAAAGATGTAATCACTAGTACATCTCGACA
 TACAACATATCCAATGTAATCTCACAATGGTATTTTCGGGAGGATAGGATATACACAAATTAAATTTATATATAGAGA
 GAGAGAGTGTGTTTTAGAGTTGCACCGTTGTGCTTTGATAAAAATACAGAATTTTATGTATAAAAAATTACACAGGTTA
 TTTCTTTATAATTTAACATCTAATAAAGCATCTTGCTTCAGTTGGTGAAAGTTTATTTCCAATAAAAAATATGTCAATTTA
 GTGGTATATATGTCACTCCTCACCGAAGACCAAACTATATGGTGGTATGCATCTATTGATTTATTGTAAATAAAAATAT
 ATCTGAGTTTGCTTGATAATAAATTATTATGACGTGTTTTAATTTGCACTATCAAGAAAGACTTTATTACAATTTTCAATC
 ATTAAGCTCGAAAACTCTAATCTTATGTATAACAAAACAACACAAGGGAAAAATTGCCATAGCTCTTTGGTGAATCAAA
 TTCCCAACTTGTGACGTGTCTCAAGTATAAATAAAAGAAAAATTGTGTGTGAGAGAAATGAAAAATAAACATTTATGA
 AGACATAGTGTGTTGTTGGGTGAAAAAGTGATACTTTTGATTTTTTTTTAAAAAGGGGACAGTGTTTTTTTATTGTTTT
 TTCTCTATGGCTGTCCCATCCCTCACCTACCCACTCAAAATTATTCTATTTAGATAAAAGACTCTTCTTGTTGTATGA
 AAAGTCATACCTAGGAAAAGAGTAGGAAAAAGGATAATTTTTGATATATTTATTTATGTCCTTAACCTTTAATATTAA
 TGAGTAACTTTTGAATTTGTATAAAGTTAAATTAATTAGATATATCAATCGTACGTAACATAAAATTAGATGAGGGTATA
 AAAGCCCTACATGAGGTGCATGTATTGTTATATACATTACGTTATAACACAAACAGTTGAGGATTGCACAACAACTTGTG
 ATCATCGGATGCGAGAAGCTCGTAAATGGCCGAAATAAATAACACATACCAAGTATAACCCGACAAGTGGGATGTGTAT
 ATAGACCTTATCACTATCTTGTGGGATAGAGGGTTGTTTCGATAGACCTCAGCTCAAGTAAACATAGGGAAAAAATAC
 CCTAGAGAAAAGGAAAAATGGAACGGATTTCATTTCTTTCACACTGTTACTACTATGAAAAATAATGGTCAAAATCGAACTC
 AATTGTTAATTGCCCAATATAGGATTGACTACTGATTCTTAAGGAACGCGTTTTCACTACCTTTTGAACCACAAAAAAT
 GGACAATTCCTTTCTTAGAGACACATACATAAAACGAGCTGAAATGTGTTTTGTTCTACCAGAGTTGTTTCAGATTGC
 ATCAATTTCACTTTCACATTTCTGTTTTTTTTTAATTATTTAAAGTTGCCAGAGCTTTGAATCTGATTTAAATTTTGATTT
 GTTAAACATTGAGTGTGTTTTTAATTAGGTTTCGTGGACCTTAATAATAGGTCACCTGTCAGAAATGATTTACAAAACT
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 TAGAATGACATAAATAAATCATTTTAATAAAATTTGATCAAAATGCTTATTACTATTTTCAAAAAATATATACACTAAGG
 GCCCGTTGGATGGGCTTAAAAAAGCAGCTTTAAAAAAGTACTTTCGAAAGGCGCTGAACTTATTTTTTGAATAAG
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 TTATTTTTCTTGCCCTTAATATTTTTATACAATCTCAAAATACGACATAACCCTAACATCTTTTTCTCCCATTTTTTCC
 TTTTCACGTTTGACATAGCAACTTCAGCACTTTTATCCAAACACATAACTGCTTATTTTAAAAATAAGTTTCAGCACTTT
 CAAAAGTACTTTTTTAAAGCTGCTTTTATTAAGCCCATCCAAACGGGCCCCAAAATTGCTAATGTTTGCTCTTTCTATTCT
 TCAAACTCCGTAATTTTAAAGAAAAATTTGCTAATGATAGGTCACTTTTAACTAAATAATTATAAATTTGGGTAGAAAT
 TATTTATCATTTTAAAGCTTTTTTAATTTTGAGTCTTCTCCCTAATTAAGACCTTCCCTCTTGCTTCAATTATTTAACT
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 TATGGAGAAAAAATATTATAGGAGAAAAATAATTAATTTTAATTCATGAATATGCTTAAATATGCAACTCATTTTGCTT
 ATATATATCAAAATTAACCTCTGTTCCCTTAACTTTTTCTATGAAGATACATTTTAATTTATTTGATGAGGTTAGTTTG
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 ATATAAGAAATAATTATGATGAAATTTATATATGTTTTATGCAGAGATTTATTACGCATTGTTTACTTGGGTTATGTATT
 ACTTATTTTATCTTTTATCAGAATGTAATAATTAATTCATTAATAAGAAATCCAATTTCTGTAAATTTCAAAATACAAACAAT
 AACATTTTCAAGAACGATTTTTTGGCCAAAGAATATACAGTAAACATATTTATGATATGGTAGGTCTCTTAGTAAATTGAC
 CAACAAGGATTGTGGTGGAGTGGGAAATACTCTTAAATACTTCACCAAGAGGTCTCCAATTTGAGCCCTGAATACGAAA
 TCGTCTTTGTTAGTATATACCTAACCTAATACAAAAATTAGTATAATTAGCTTCACAGCTAAAACTTTGTGACCTGTA
 AGTCACGCGAGGACAAATTTACCGTAACACCAACTTATTCATGATATAATTGTCCCTTTTAGCACGGTAATAATGAGGTG
 GGTAAGAAATTTATTTAGTGGGCTTTCTACACCCACCTTATTCTCTTGCTTCAATTTATGAATTGAAGAAGTAATG
 AAAAAACAGACTCCATTGGATAAAGGACAGTTTGCACACAGCTGTAAACAATTTAGAGCACTAGCAAAATAGAGAGAGT
 TTTGAGAGAAATTTTTGTTTGCAAATTAATCTTAACCTTCAGCAGGTAATAAAGTTCTTAACCTGAGACTATTTGAAGA
 TATATTTGTAAAGAAATCATTTTGTGTGTTCTTGTGTTTGTCTTTGTCAGATTGAGAAATG

FIGURE 9



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